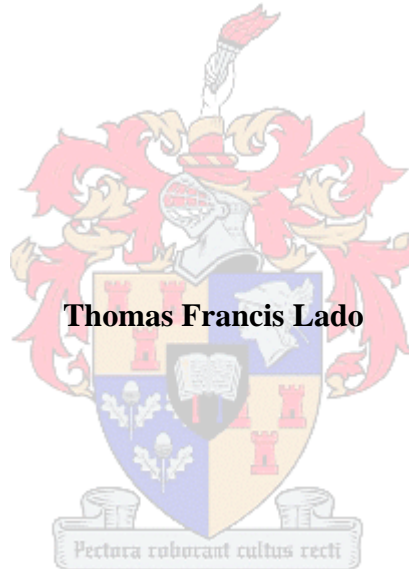


**Molecular Ecology of Introduced Species in South Africa:
The bud gall-forming wasp *Trichilogaster acaciaelongifoliae* and
the Argentine ant *Linepithema humile*.**



Dissertation submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy in the
Department of Botany and Zoology,
Stellenbosch University
South Africa

Supervisors:

Dr Bettine Jansen van Vuuren

Prof Steven L Chown

November 2008

Declaration

I, Thomas Francis Lado, hereby declare that this dissertation is my own original work and has not previously been submitted for any degree or examination at any University. Specimens were collected under permits issued by Cape Nature, Western Cape (Permit no. 001-204-00037), Department of Economic Development and Environmental Affairs, Eastern Cape (Permit no. WRO 32/ 06 WR) and by the KwaZulu-Natal National Wildlife Permits Office (Permit no. 1237/ 2006 and 934/ 2007). Written permits were not required for Gauteng and Mpumalanga Provinces.

.....

Thomas Francis Lado

On this.....day of2008

Copyright © 2008 Stellenbosch University

All rights reserved

For my deceased father

**“What lies behind us and what lies before us are small matters
compared to what lies within us”**

Ralph Waldo Emerson

Abstract

Introduced species displace native species and alter ecological communities, affect agriculture as well as human health and are economically costly to eradicate. Long term monitoring of introduced species including the documentation of levels of genetic variation is therefore of the utmost importance. This study investigated the distribution of genetic variation in two introduced species distributed across South Africa the bud gall-forming wasp *Trichilogaster acaciaelongifoliae* and the Argentine ant *Linepithema humile*.

The bud gall-forming wasp was introduced into South Africa as a biological control agent to curb the spread of the invasive long-leaved wattle *Acacia longifolia*. In addition to the intended (target) host, the bud gall-forming wasp has also colonised *A. floribunda*, a non-invasive ornamental plant. Limited genetic variation was found across South Africa based on the mitochondrial DNA cytochrome oxidase subunit I (COI) gene. Only 3 haplotypes characterized 53 individuals collected from 23 localities (nucleotide diversity $\pi = 0.002 \pm 0.001$, haplotype diversity $h = 0.482 \pm 0.045$). No significant partitioning of genetic variation was found across South Africa including between host plants (target host = *A. longifolia*, non target host = *A. floribunda*) or between the core (sites of introduction) and edge (naturally dispersed) sites ($\Phi_{ST} = 0.094$, $P = 0.288$). The limited genetic variation and the absence of significant genetic structure are congruent with patterns described for many other introduced species and may suggest that propagule pressure plays only a minor role in species establishment and spread of the gall-forming wasp across South Africa.

Mitochondrial and nuclear markers were used to describe the distribution of genetic variation within Argentine ants across their introduced range in South Africa. For the mitochondrial DNA, low genetic diversity was found for the COI gene with only five

haplotypes, separated by single mutational changes, characterizing 101 specimens from 35 localities (nucleotide diversity $\pi = 0.001 \pm 0.001$, haplotype diversity $h = 0.151 \pm 0.048$). Notwithstanding the low levels of genetic diversity, mitochondrial variation was significantly structured ($\Phi_{ST} = 0.54$, $P < 0.001$) across the landscape. In contrast, microsatellite analyses of 230 ants from 23 localities, employing six polymorphic microsatellite markers, revealed a relatively high amount of genetic diversity ($H_E = 0.51 \pm 0.22$). Significant population structure was similarly evident ($R_{ST} = 0.14$, $P < 0.001$) with the localities of Elim2, Porterville2 and Bloemfontein2 clustering as a distinct population from the remainder of the localities. Importantly, individuals from these localities also had a unique mitochondrial haplotype and, when taken with the nuclear results, may indicate the occurrence of more than one introduction event (and possibly more than one colony) in South Africa. This is further underscored by the presence of unique microsatellite alleles in these three populations.

In an attempt to establish the source populations for the introduction of Argentine ants into South Africa, mitochondrial cytochrome b sequences were generated for a subset of ants representing the two major genetic clades across South Africa. A comparison with the published data from across the world including the native range of the Argentine ant in South America grouped Argentine ants from South Africa with three potential source populations namely Ocampo and Rosario in Argentina and Passo do Lontra in Brazil.

The results of this study underscore the role of human-mediated dispersal in shaping the levels of genetic variation in both species. Human-mediated dispersal can lead to genetic homogenization across populations.

Opsomming

Indringer spesies verplaas of verander ekologiese gemeenskappe, beïnvloed landbou asook menslike gesondheid en is ekonomies duur om te verwyder. Langtermyn monitering van indringer spesies asook die dokumentasie van genetiese variasie is dus baie belangrik. Hierdie studie bestudeer die verspreiding van genetiese variasie in twee indringer spesies wat regoor Suid-Afrika voorkom, naamlik die kroongal-vormende wespe *Trichilogaster acaciaelongifoliae* en die Argentynse mier *Linepithema humile*.

Die kroongal-vormende wesp is na Suid Afrika gebring as biologiese beheeragent om die verspreiding van indringer lang-blarige wattle *Acacia longifolia* te beveg. Die kroongal-vormende wespe het sowel die teiken spesies, asook *A. floribunda*, 'n nie-indringer ornamentele plant gekoloniseer. Beperkte genetiese variasie is gevind regoor Suid Afrika gebasseer op die mitochondriale DNA sitokroom-oksidase subeenheid I (COI) geen. Slegs 3 haplotipes karakteriseer 53 individue van 23 bevolkings (nukleotied diversiteit $\pi = 0.002 \pm 0.001$, haplotiep diversiteit $h = 0.482 \pm 0.045$). Geen beduinde groepering van genetiese variase is gevind regoor Suid Afrika nie ($\Phi_{ST} = 0.094$, $P = 0.288$). Hierdie bevinding geld onafhanklik van die gasheer plant (teiken gasheer = *A. longifolia*, nie-teiken gasheer = *A. floribunda*). Ook is geen beduidende genetiese groepering gevind tussen die sentrale (plek van inisiele blootstelling) en perifêre (natuurlik verspreide) lokaliteite nie. Die kleinskaalse genetiese variase en die afwesigheid van beduidende genetiese struktuur wat hier gevind is, verskil van die patrone wat voorheen vir baie ander indringer-spesies beskryf is. Dit mag daarop dui dat 'propagule' druk slegs 'n klein rol speel in spesies-vestiging en verspreiding van die gal-vormende wespe regoor Suid-Afrika.

Mitochondriale asook kern merkers is gebruik om die verspreiding van genetiese variasie in Argentyse miere in Suid Afrika te beskryf. Vir die mitochondriale DNA is lae genetiese variase gevind vir die COI geen, met slegs 5 haplotipes, gedifferensieer deur enkele mutasie veranderinge wat 101 monsters van 35 lokaliteite karakteriseer (nukleotied diversiteit $\pi = 0.001 \pm 0.001$, haplotiep diversiteit $h = 0.151 \pm 0.048$). Desondanks die lae genetiese variasie, is gevind dat mitochondriale variasie beduidend gestruktureerd is ($\Phi_{ST} = 0.54$, $P < 0.001$) oor die landskap. Hierteenoor het mikrosatelliet analyses van 230 miere van 23 lokaliteite, deur gebruik te maak van ses polimorfiese mikrosatelliet merkers, 'n relatiewe hoë hoeveelheid genetiese diversiteit aangedui. Beduidende bevolkingstruktuur was ook gevind ($R_{ST} = 0.14$, $P < 0.001$) in die areas Elim2, Porterville2 en Bloemfontein2 wat saam groepeer as eiesoortige bevolkings vergeleke met die res van die areas. Ook van belang is dat individue van die areas 'n unieke mitochondriale haplotipe besit, en in kombinasie met die kern resultate, mag dit die voorkoms van meer as een blootstellingsgeleentheid (en moontlik meer as een kolonie) in Suid Afrika aandui. Hierdie bevinding word verder beklemtoon deur die teenwoordigheid van unieke mikrosatelliet allele in die drie bevolkings.

In 'n poging om die oorsprong van die oorsprong-bevolking vir die blootstelling van Argentyse miere in Suid Afrika vas te stel, is mitochondriale sitokroom b volgordes gegenereer vir 'n substel miere wat die twee hoof genetiese klades in Suid Afrika voorstel. Vergelyking met gepubliseerde data van regoor die wêreld, insluitende die endemiese gebied van die Argentyse mier in Suid Amerika, het die Argentyse mier van Suid Afrika met drie potensiele oorsprong-bevolking verbind, naamlik Ocampo en Rosario in Argentina en Passo do Lontra in Brazil.

Acknowledgements

I am indebted to Prof Steven L Chown and Dr Bettine van Vuuren for their supervision, critical and constructive discussions, guidance and assistance during the course of this study. Without their valuable insights I would not have been able to reach the final stages of my study.

This project was funded through a DST-NRF Centre of Excellence for Invasion Biology (C•I•B) grant to Prof Chown. Bursary support from the C•I•B is gratefully acknowledged.

Specimens were collected with permission from Cape Nature (Permit no. 001-204-00037), Eastern Cape Department of Economic and Environmental Affairs (Permit no. WRO 32/ 06 WR), KwaZulu-Natal (Permit no. 237/ 2006 and 934/ 2007), Gauteng and Mpumalanga Nature Conservation Agencies. Numerous farmers and private forest owners are thanked for allowing us to work on their properties.

A number of institutions and people contributed to the successful completion of this study. My colleagues from both the C•I•B and Spatial and Conservation Ecology Group (Ruan Veldtman, Ndivhuwo Luruli, Charlene Janion-Scheepers, John Terblanche, John Wilson, Dian Spear, Elrike Marais, Antoinette Botes, Ulrike Irlich, Jeanne Gouws, Michelle Greve, Meagan Stevens, Keafon Jumbam, Ethel Phiri, Natasha Mothapo, Suzaan Kritzing, Serban Porches, Nigel Andrew, Cang Hui, Jacques Deere, Jacko Klock, Brent Sinclair, Richard Mercer and Catherine Parr), and the Evolutionary Genomics Group (Liezl Mortimer, Sandi Willows-Munro, Jane Deuve, Clement Gilbert, Andrew Bowkett, Adriaan Engelbrecht, Nina du Toit, Prince Kaleme, Woody Cotterill, Sophie von der Heyden, Aurora Ruiz-Herrera, Conrad Matthee, Rauri Bowie, Savel Daniels, Victor Rambau, Vidya Chakravarthy, Andrew Bowkett and Sampath Lokugalappatti) as well as Nigel Andrew of the University of New England in

Australia, deserve my thanks and appreciation for their support, valuable discussions, help with sample collection and advice during the course of this study.

I am grateful to all of my friends and former housemates for their unswerving support particularly Timothy and Ruth Jost, Tom Fluri, Simeon Hengari, André de Villiers, Cristiano Lana, Fred Aguesse, Ibo Hander, James Rodger, Mawethu Nyakata, Sebataolo Rahlao, Lebohang Sekhokoane, Benis Egoh, Shelley Johnson, Mark van Kleunen and Vanessa Pasqueleta.

Finally, thanks to my family for their understanding for me to be away from them for such a long time.

Table of Contents

Molecular Ecology of Introduced Species in South Africa:	1
The bud gall-forming wasp <i>Trichilogaster acaciaelongifoliae</i> and the Argentine ant <i>Linepithema humile</i>	1
Declaration	I
For my deceased father.....	II
Abstract.....	III
Opsomming	V
Acknowledgements	VII
Table of Contents	IX
List of Figures.....	XI
List of Tables.....	XIII
List of Abbreviations	XV
Chapter 1: General Introduction	1
1.1 Genetics of alien biological introductions	1
1.2 Study species: Biology and background to introductions	2
1.3 Molecular phylogeography and evolution of alien introduced species	6
1.4 Factors affecting genetic variation in introduced species.....	7
1.5 Objectives	8
Chapter 2: Distribution of genetic variation of the Bud gall- forming wasp in South Africa	10
2.1 Introduction	10
2.2 Research questions and hypotheses:.....	12
2.3 Materials and methods.....	13
2.4 Results	16
2.5 Discussion.....	19

Chapter 3: Distribution of genetic variation in Argentine ants across South Africa.....	27
3.1 Introduction	27
3.2 Research questions	29
3.3 Material and methods	30
3.4 Results	42
3.5 Discussion.....	58
3.6 Lessons from this study for the control of Argentine ants in South Africa.....	66
Chapter 4: Summary and avenues for future research.....	67
4.1 <i>Trichilogaster acaciaelongifoliae</i> (Chapter 2)	67
4.2 <i>Linepithema humile</i> (Chapter 3)	68
References	71
Appendix	90

List of Figures

Fig. 1 Map of South Africa indicating the collection localities for <i>T. acaciaelongifoliae</i> . Provincial boundaries are indicated. Core sites are indicated in green filled circles and edge sites in yellow filled circles.	14
Fig. 2 Statistical parsimony network created in TCS depicting the relationships between haplotypes. Oval sizes are drawn according to the number of individuals (given in brackets).	18
Fig. 3 Rarefaction curve of <i>T. acaciaelongifoliae</i> haplotypes in South Africa based on 23 sampling localities. Number in front of the curve refers to the estimated number of haplotypes based on Jack2.	19
Fig. 4 Map of South Africa indicating the sampling locations for the Argentine ant, <i>L. humile</i> . The main road network in South Africa is indicated. Red circles indicate sites that were visited but where Argentine ants were not found. Localities where Argentine ants were present and for which mitochondrial sequence data are available are indicated in green. Localities where Argentine ants were present and for which both mitochondrial and microsatellite data are available are indicated in yellow.	31
Fig. 5 Statistical parsimony network generated in TCS for Argentine ants sampled from across South Africa. This network is based on 618 bp of COI sequence for 101 ant specimens. The square (haplotype LH5) represents the presumed ancestral haplotype. The sizes of haplotypes are drawn according to their respective frequencies.	43
Fig. 6 Rarefaction curve showing the observed COI haplotypes and the number of sampling localities. The estimated number of Argentine ant haplotypes based on Jack1 is given in front of the curve.	44
Fig. 7 Non-metric multidimensional scaling (NMDS) of Argentine ant genetic distances. The plot is based on 618 bp of COI gene sequences for 101 ant specimens from 35 geographic localities. Stress value S is < 0.1	45

Fig. 8 Neighbour-joining tree based on 407 bp of cytochrome b. The tree was constructed from HKY+G corrected sequence divergences. South African haplotypes are indicated in bold. Two of the South African haplotypes grouped with those from Argentina, Brazil, New Zealand and Australia (indicated in blue). Refer to Table 4 for abbreviations.....	48
Fig. 9 The haplotype network constructed in TCS. Four haplotypes could not be connected within the 95% probability interval and these are shown on the side. Sizes of haplotypes are drawn according to their respective frequencies. Number of mutational steps between the haplotypes is indicated. South African haplotypes are shown in bold. Two of the South African haplotypes grouped with haplotypes from Brazil, Argentina, New Zealand and Australia and are shown in blue Refer to Table 4 for abbreviations.....	49
Fig. 10 Rarefaction curve of observed microsatellite allele variation and the number of localities sampled. Jack1-based estimated number of alleles is given in front of the curve.	51
Fig. 11 Non-Metric Multidimensional Scaling of genetic distances of Argentine ants in South Africa showing the clustering of populations from Elim2, Porterville2 and Bloemfontein2 (Red). Stress value (S) was < 0.150.	54
Fig. 12 Moran's I correlogram showing Moran's I coefficients plotted against geographic distance for Argentine ant populations in South Africa based on microsatellite data. Black squares represent autocorrelation coefficients of the Moran I statistics.....	58
Fig. 13 Map of British troop movements during the Anglo-Boer War redrawn from Vernon (1985). Path followed by the British troops is indicated by the truncated black line. Railway lines and important towns at the time are indicated.	62

List of Tables

Table 1 Sampling localities (including geographic coordinates) for <i>T. acaciaelongifoliae</i> . Sample sizes, core and edge localities are indicated, as well as host plant included per locality. The occurrence of the three mitochondrial DNA haplotypes is indicated.	17
Table 2 Two polymorphic sites (positions 63 and 255) resulted in three haplotypes. The frequencies of haplotypes are indicated (number of specimens are indicated in parenthesis)...	17
Table 3 List of localities, geographic positions (latitude and longitude in decimal degrees, altitude in metres), composition of the observed COI and cytochrome b haplotypes as well as localities (= populations) for which microsatellite data were included.....	33
Table 4 Argentine ants were included from across the world in an attempt to identify putative source populations for introduction into South Africa. Haplotypes are based on 407 bp of cytochrome b data. The South African localities are shown in bold. The South African haplotypes indicated here are based on 738 bp of sequence data.....	37
Table 5 Argentine ant haplotypes identified for the COI gene. Polymorphic nucleotide positions, distribution of haplotypes across South Africa and number of specimens characterized by haplotypes are indicated.	43
Table 6 Populations and samples (n) included in the microsatellite analyses. Mean number of alleles (A_O), observed (H_O), expected heterozygosities (H_E) and R_{ST} are reported.	50
Table 7 Null allele frequencies for four loci and 12 populations of Argentine ants in South Africa. The negative frequencies are a result of the algorithm used (see discussion).....	52
Table 8 Comparisons between observed and adjusted allele frequencies for loci in populations where null alleles were detected. Significance was determined through a Chi square test with degrees of freedom (DF) and significance indicated (Ns = non significant).	53
Table 9 Rare alleles and their frequencies per Argentine ant population in South Africa. Unique alleles are listed per group.	55

Table 10 Heterozygosity excess and P-values of Argentine ant populations in South Africa.	
Bottlenecked populations are indicated by *	57
Table 11 Allele frequencies of Argentine ants in South Africa	90

List of Abbreviations

A = Adenine nucleotide

ABI = Applied Biosystems, Inc.

A. floribunda = *Acacia floribunda*

A. kollari = *Andricus kollari*

A. longifolia = *Acacia longifolia*

AMOVA = Analysis of Molecular Variance

ANOSIM = Analysis of Similarity

A. quercuscalicis = *Andricus quercuscalicis*

bp = Base Pairs

COI = Cytochrome Oxidase Subunit I

C = Cytosine nucleotide

Cyt b = Cytochrome b

G = Guanine nucleotide

HWE = Hardy-Weinberg Equilibrium

LD = Linkage Disequilibrium

Mt DNA = Mitochondrial Deoxyribonucleic Acid

NMDS = Non-Metric Multi-Dimensional Scaling

NS = Non Significant

PAST = Palaeontological Statistics

PCR = Polymerase Chain Reaction

r = Coefficient of Relatedness

R = Analysis of Similarity measure

S = Stress Measure

SPAGeDi= Spatial Pattern Analysis of Genetic Diversity

T = Thymine nucleotide

TPM = Two Phase Mutation Model

Chapter 1: General Introduction

Alien invasive species are taxa deliberately or unintentionally introduced outside their native range that colonize, establish and spread in new environments in such a way that they displace endemic species (Di Castri *et al.* 1990, Richardson & Pyšek 2006, Zee & Holway 2006). Such invasions often culminate in significant environmental, conservation and economic crises (Mack *et al.* 2000, Pimental *et al.* 2000, Christian 2001, Sakai *et al.* 2001, Olson 2006). Purposely introduced species are released because it is believed that they will benefit the environment in some way for example as biological control agents (Slade & Moritz 1998, Hill *et al.* 2000). In addition to benefiting the environment, many species have been introduced to benefit humans directly (like animals and plants for food) (Diamond 2002). To adequately manage and control alien species, studies of risk assessment and monitoring of factors associated with success and failure of biological invasions are of the utmost importance (Loeb 1994, McEvoy 1996, Louda *et al.* 1997, Corry & Myers 2000). Included here is information regarding the genetic structure of species across their native and introduced ranges, whether genetic variation is geographically structured, and the source of introduction which is often unknown for unintentionally introduced species. The aim of this study is to contribute genetic information on alien species in South Africa. This kind of information, which is mostly lacking, is essential in improving control measures for species through biological, chemical and cultural approaches.

1.1 Genetics of alien biological introductions

Ecological approaches have identified many of the factors associated with successful invasions such as a high tolerance to environmental heterogeneity and the ability to reproduce both asexually and sexually (Groves & Di Castri 1991, Pappert *et al.* 2000, Sakai *et al.* 2001, Johnson & Starks 2004, Kolbe *et al.* 2004, Petit *et al.* 2004). In contrast, studies investigating

the genetic aspects associated with invasion success of a species have received less attention. These kinds of information are crucial for several reasons. First, levels of genetic diversity of a species in its native range can determine how its members might respond to various biotic and abiotic factors including those encountered in introduced areas (van Driesche & Bellows 1996, Falk *et al.* 2001, Sakai *et al.* 2001). Secondly, knowledge about the geographic distribution of genetic variation across the species' native range can aid in understanding (and predicting) the nature and origin of the genetic differences between populations in the introduced range (Merrel 1981, Franks *et al.* 2004, Miura 2007). Third, comparative phylogeographic studies of invasive species in their introduced versus native ranges may highlight special genetic features of the colonists (Parsons 1983, Tsutsui *et al.* 2000, Petit *et al.* 2004). These kinds of studies provide ideal opportunities to investigate the occurrence of rapid evolution in a species following its introduction (Ferraris & Palumbi 1996, van Klinken & Edwards 2002, Frankham 2005). Fourth, understanding the patterns of population structure and variation at different scales can provide additional information about the nature of gene flow and likelihood of genetic drift (Hengeveld 1990, McDonald & Potts 1997, Schäfer *et al.* 2001, Kalisz *et al.* 2001). This genetic information can be used to infer likely vectors of dispersal and patterns of spread; information that is essential for the management of biological control agents as well as for prevention and eradication of invasive species.

1.2 Study species: Biology and background to introductions

Within South Africa, the bud gall-forming wasp, *Trichilogaster acaciaelongifoliae* and the Argentine ant, *Linepithema humile* provide models for the study of genetic aspects associated with the success of an introduced species. *Trichilogaster acaciaelongifoliae* was introduced into South Africa from Australia as a biocontrol agent for the Australian long-leaved wattle, *Acacia longifolia*. *Linepithema humile* was accidentally introduced into South

Africa with animal fodder at the turn of the previous century (~1900) and has since spread through large parts of the country.

1.2.1 *Trichilogaster acaciaelongifoliae* (Frogatt) (Hymenoptera: Pteromalidae)

The bud gall-forming wasp was introduced into South Africa to curb the spread of the invasive long-leaved wattle (Dennill 1987). The introduced specimens all came from the coastal regions of New South Wales, Victoria and Tasmania and were mainly collected from *A. longifolia* and the sally (gossamer) wattle *A. floribunda* (Costermans 1981, Dennill 1987, Wrigley & Fagg 1996). Two releases were made into the Western Cape Province in 1981 and 1982. All further releases into others parts of the country were drawn from the subsequent generations that emerged from these initial releases (Dennill 1987). The bud gall-forming wasp has spread through large parts of South Africa where its host plants *A. longifolia* and *A. floribunda* are found (although the latter is not considered an invasive in South Africa, it is also host to the wasp). *Trichilogaster acaciaelongifoliae*'s current range includes the Eastern and Western Cape Provinces, KwaZulu-Natal, Gauteng and Mpumalanga (Dennill 1987, McGeoch & Wossler 2000, Hoffman 2001, Dennill & Gordon 1990). Its establishment has been marked with varied success associated with climatic variability and also shade provided by other tree species (Dennill 1987, Dennill & Gordon 1990).

The bud gall-forming wasp is characterized by a univoltine, and occasionally bivoltine, life cycle including parthenogenic reproduction (Dennill *et al.* 1993). Males are haploid and females are diploid (Manongi & Hoffman 1995, Hoffman 2001). The sex ratio of this wasp varies according to host plant with both male and female eggs being deposited in a single gall (Noble 1940). Adult wasps with a life span of 2 – 3 days emerge between October and January reaching their peak in November (Dennill 1987).

They exhibit sexual dimorphism with males being smaller and darker than the females (Hoffman 2001). Females lay up to 400 eggs inserted in batches of 15 eggs per batch. These batches are mostly laid into immature buds and, to a lesser extent, into the vegetative parts of the host plant (Hoffman 2001). Eggs hatch in spring producing wasp larvae, which then initiate the formation of galls with up to eight larvae, each with its own chamber (Dennill 1987).

1.2.2 *Linepithema humile* (Hymenoptera: Formicidae)

Linepithema humile is one of the most successful invaders world-wide (Suarez *et al.* 1999, Silverman & Brightwell 2008) and has successfully established on all continents with the exception of Antarctica (Suarez *et al.* 2001, Corin *et al.* 2007a). Much is known about the biology and spread of the Argentine ant (Tsutsui & Case 2001, Vega & Rust 2001, Wild 2004, Silverman & Brightwell 2008) and it is agreed that in introduced ranges it is detrimental to the local diversity of flora and fauna (Skaife 1953, Christian 2001, Nauman *et al.* 2004). Within South Africa, this species was first recorded in Stellenbosch in 1908 and is said to have accidentally entered the country in forage imported from South America (Skaife 1953, Prins 1978). Previously, the distribution of the Argentine ant in South Africa was not well known but it was reported to be extremely common throughout the south-western Cape area (Skaife 1953, Slingsby & Bond 1981). However, data gathered by Luruli (2007) as well as in the present study indicate the species to be widely distributed throughout South Africa.

The native range of the Argentine ant covers north-eastern Argentina, Brazil and southern Paraguay along the Paraná drainage system (Tsutsui *et al.* 2001, Vega & Rust 2001, Wild 2004). Its distribution, both in its native and introduced ranges, has been associated with high humidity and mild winter temperatures, disturbance and specific soil and vegetation types

(Markin 1969, Krieger & Keller 1999, Slingsby & Bond 1981, 1983; Witt & Giliomee 1999, Holway *et al.* 2002, Walters & Mackay 2004, Wild 2004).

The Argentine ant has low levels of genetic diversity across its introduced ranges and occurs almost exclusively as single large colonies (Tsutsui *et al.* 2000, Corin *et al.* 2007b but see Ingram & Gordon 2003, Buczkowski *et al.* 2004). This is in contrast to the pattern across its native range where ants display a multi-colonial social structure with aggression between workers of different nests. In addition, it also coexists with other ants in species-rich communities (Krieger & Keller 1999, Suarez *et al.* 1999, Tsutsui *et al.* 2000).

Argentine ant workers are aggressive and although they do not have stings they bite when provoked. Argentine ants also employ chemical defences when provoked. Queens and drones are winged and reproductive (Skaife 1953, Passera & Keller 1994, Krieger & Keller 1999, Krieger & Keller 2000, Nauman *et al.* 2004). The workers are monomorphic, whereas the queen is almost twice the length of worker ants. The males and queens mate within the colony, and the queens disperse by budding rather than nuptial flights. This information is important for investigating expected patterns of isolation-by-distance in natural populations (Passera & Keller 1994, Vega & Rust 2001). Under reduced chances of mating due to the presence of only a few females, males may disperse to other colonies (Passera & Keller 1994, Nauman *et al.* 2004). The queens lay a large number of eggs which are gathered and cared for by the workers although queens may infrequently also care for eggs (Skaife 1953, Nauman *et al.* 2004).

1.3 Molecular phylogeography and evolution of alien introduced species

Natural or native populations of a species are expected to exhibit varying degrees of genetic diversity and differentiation depending on the relative roles of factors such as isolation-by-distance, mutation, natural dispersal and selection (Gardner & Snustad 1984, Epperson 2003, Lowe *et al.* 2004). Intuitively it would be expected that (recently) introduced populations of a species would be characterized by lower levels of genetic diversity when compared to the species in its natural range (see for example Baker *et al.* 2003, Grapputo *et al.* 2005, Lloyd *et al.* 2005, Zayed *et al.* 2007). This has not always been the case with several studies that have compared levels of diversity between introduced and native populations showing no differences between these two ranges. For example, Johnson and Starks (2004) found no significant reduction in mitochondrial DNA diversity of the invasive wasp *Polistes dominulus* in the United States of America relative to its European native range.

It is difficult to *a priori* predict the amount of genetic structuring present in introduced alien species. Whereas population genetic structuring across introduced ranges may arise as a result of directional selection, lack of gene flow between geographically distant populations, mutation, host specificity and isolation-by-distance or genetic drift (Filchak *et al.* 2000, Carrol *et al.* 2007, Frankham *et al.* 2002, Lee 2002), many species show very little genetic structuring across their introduced ranges. For example, Goodisman *et al.* (2001) found significant allelic differentiation at three polymorphic microsatellite loci of the introduced wasp, *Vespula germanica* across Australia. Likewise, Lakatos *et al.* (2003) found significant genetic differentiation between introduced populations of the leaf miner moth *Parectopa robiniella* in North America and Europe. In contrast, Kim *et al.* (2003) found no genetic differentiation in the introduced populations of the diamond-back moth *Plutella xylostella* in Korea. Similarly,

no genetic differentiation was observed in the introduced populations of springtail species on Marion Island (Myburgh *et al.* 2007).

Even within a single species it is impossible to extrapolate results from one geographic area to another with much certainty. For example, introduced Argentine ant populations across the world show almost no genetic differentiation among nests (Dreier *et al.* 2005, Corin *et al.* 2007b) with substantial gene flow (Krieger & Keller 2000). However, significant partitioning of genetic variation has been reported for this species in Jasper Ridge Biological Preserve in California (Ingram & Gordon 2003) and in the Northeastern USA (Buczkowski *et al.* 2004).

1.4 Factors affecting genetic variation in introduced species

Many factors, either singly or in concert, shape the amount of genetic variation in introduced species (Sakai *et al.* 2001, Lee 2002, van Klinken & Edwards 2002). Variation may be reduced through population bottlenecks (with concomitant genetic drift in small populations) typically expressed during introduction events. Similarly, small population sizes (although this is not confined to small numbers only) may facilitate local adaptation in response to selection associated with the new environmental conditions thereby reducing variation (Allendorf & Lundquist 2003). In contrast, a high propagule pressure (including both the number of individuals introduced and the number of release events) can result in the alien species having high genetic variation (Johnson & Starks 2004, Kolbe *et al.* 2004, Miura 2007). Multiple introductions from different source populations will also increase variation. In addition, hybridization between individuals from different native populations may even cause introduced populations to have higher genetic variation compared with native populations (Ellstrand & Schierenbeck 2000, Allendorf & Lundquist 2003, Miura 2007).

Changes in genetic composition may cause shifts in the behaviour of a species in its introduced range (Money & Cleland 2001). For example, Pickett & Wenzel (2000) found that introduced populations of the paper wasp *P. dominulus* in the USA display changes in life history traits such as higher productivity and survivorship relative to native populations. Similar examples have also been reported for the fire ant *Solenopsis invicta* where the polygyne form of this species is more widely distributed in the introduced range compared to the distribution in the native range (Mescher *et al.* 2003, Shoemaker *et al.* 2003). Also, in contrast to their natural behaviour, Argentine ants in introduced ranges are unicolonial and lack aggression between nests (Tsutsui *et al.* 2000, Corin *et al.* 2007b) possibly due to the loss of genes associated with recognition cues (Giraud *et al.* 2002 but see Pedersen *et al.* 2006 who argues that these differences are largely the result of colony size).

1.5 Objectives

The main objective of this study is to provide genetic information for two species introduced to South Africa; the bud gall-forming wasp (*T. acaciaelongifoliae*) and the Argentine ant (*L. humile*). Broad research aims are given below. More specific questions and hypothesis are provided in Chapters 2 and 3.

1.5.1 Research aim for *T. acaciaelongifoliae*

1. To describe the spatial distribution of genetic variation within *T. acaciaelongifoliae* across its South African distribution range.

1.5.2 Research aims for *L. humile*

1. To describe the spatial distribution of genetic variation within *L. humile* across its South African distribution.
2. To establish whether *L. humile* constitutes a single supercolony in South Africa as observed in other parts of the world.

3. To determine the source population(s) for the Argentine ant introduced into South Africa.

Chapter 2: Distribution of genetic variation of the Bud gall-forming wasp in South Africa

2.1 Introduction

The use of biological control agents has not always been met with success. Several cases have been documented where biocontrol agents failed to curb the spread of target species (Myers 2000) or where bio-control agents underwent a host-shift to non-target or non-intended species (Louda *et al.* 1997, Corry & Myers 2000, Freckleton 2000, Myers 2000). The reasons behind these failures may include factors such as the Allee effect (Grevstad 1999, Liebhold & Bascompte 2003, Lockwood *et al.* 2005, van Kleunen & Johnson 2005, Ward & Johnson 2005, Deredec & Courchamp 2007), host plant quality including age, size and nutrient levels (Alstad 1998), founder effects (Liebhold & Bascompte 2003), population bottlenecks (Hufbauer *et al.* 2004) and/or genetic systems such as haplodiploidy (Packer & Owen 2001).

Haplodiploid hymenopterans are typically characterized by reduced levels of genetic variation (Ayala 1976, Crozier 1977, Graur 1985). This is because heterozygosity is restricted to the diploid sex (females) resulting in reduced recombination and rapid evolution (Crozier 1977, Hedrick & Parker 1997). Although moderate to high levels of genetic variation, compared to their native ranges, have been reported for certain introduced hymenopteran species such as the primitive sawflies of the genus *Cephalacia* and the European paper wasp *P. dominulus* (Boato & Battisti 1996, Johnson & Starks 2004), these are typically associated with populations that comprise multiple introductions or where hybridization occurs among different populations or even species (Allendorf & Lundquist 2003, Suarez & Tsutsui 2008).

The bud gall-forming wasp *T. acaciaelongifoliae* is native to Australia where it occurs on several *Acacia* *sp.* including *A. longifolia* and *A. floribunda* (Noble 1940, Dennill 1987). It was introduced to South Africa as a biocontrol agent to curb the spread of the highly invasive

A. longifolia (long-leaved wattle) (Dennill 1987); this species poses a threat to water courses and the fynbos biome (Boucher & Stirton 1978, Macdonald *et al.* 1985). *Trichilogaster acaciaelongifoliae* larvae release a substance that causes the flowering bud to develop into galls instead of seeds thereby reducing seed production (Dennill 1987). In 1981, 1466 females collected from four localities across New South Wales were released in the Western Cape Province in equal proportions at Banhoek, Vergelegen and Eerste Rivier and allowed to spread naturally (Dennill 1987). In 1982, a second batch of approximately 14791 females were imported from nine localities; these included the original sites of capture in New South Wales as well as six localities from Victoria and Tasmania in Australia (Dennill 1987). These wasps were mixed and released at four additional (experimental) sites in the Western Cape namely Stellenbosch Mountain, Die Boord, Klein Drakenstein and La Motte (Dennill 1987). The first, second and third generation wasps that emerged from these experimental sites were subsequently released at 17 sites throughout the Western Cape Province (during 1983), 49 sites in the Eastern Cape Province (during 1984) and at 18 sites in KwaZulu-Natal (during 1985) (Dennill 1987). This species have since spread across South Africa and now occurs throughout the entire range of *A. longifolia*. In addition, *T. acaciaelongifoliae* has expanded its host range locally and now also parasitizes the sally wattle *A. floribunda* (Wrigley & Fagg 1996, McGeoch & Wossler 2000) which is a non-invasive ornamental plant in South Africa.

Although the bud gall-forming wasp has spread across the entire range of both acacias in South Africa and is estimated to reduce seed production in *A. longifolia* by about 80% in some areas (Dennill 1987), low levels of genetic diversity may make haplodiploid organisms particularly vulnerable to processes that reduce genetic variability including introductions but also connectivity among populations or other founder events (Zayed & Packer 2005). However, information pertaining to the genetic variation present in the wasp has largely been

lacking. This information might prove vital to guide future introductions should the ability of the wasp to reduce seed production of the host trees decline in the future.

Introduced populations of the bud gall-forming wasp are expected to show low levels of genetic variation across its range. To document genetic variation in the introduced *T. acaciaelongifoliae* and determine whether variation is geographically structured, a portion of the cytochrome oxidase subunit I (COI) gene was sequenced. This gene, which is situated in the mitochondria and therefore maternally inherited, is frequently used to document variation at the population level (Smith 2005, Ros & Breeuwer 2007). This gene region was further selected since it is the region of choice for the barcoding of vertebrates and invertebrates (Hebert *et al.* 2003). The use of this gene is, however, not without controversy with various authors suggesting that it may not represent a strictly neutral marker (see for example Ballard & Kreitman 1995, Confalonieri *et al.* 1998, Blier *et al.* 2001, Bazin *et al.* 2006). Mitochondrial DNA as a non-neutral molecule would imply that certain haplotypes would be favoured under different environmental conditions thereby reducing genetic variation through selection. If a specific haplotype has a significant selective advantage, mitochondrial DNA variation may rapidly be reduced until a single haplotype characterizes all specimens.

2.2 Research questions and hypotheses:

The following specific questions were posed;

- Given that the distribution of *T. acaciaelongifoliae* in South Africa spans areas that were initially considered climatically unsuitable for the species, is there any evidence of geographic diversification such that the populations are genetically distinct?

- Given that *T. acaciaelongifoliae* oviposit eggs and preys on both *A. longifoliae* and *A. floribunda* as host plants, are there any genetic differences in wasps associated with the different host plants?
- Are populations at the core (introduced sites) genetically distinct from those at the periphery (naturally dispersed sites) across the distribution range?

The above mentioned questions rotate around two hypotheses;

- Individuals of *T. acaciaelongifoliae* from different geographic or climatic zones, host plants and distribution range across South Africa are genetically distinct.
- Observed levels of genetic variation in *T. acaciaelongifoliae* in South Africa is a reflection of founder effects and human-mediated dispersal.

2.3 Materials and methods

2.3.1 Sample collection.

Field sampling was done at 23 localities covering the distribution range of the bud gall-forming wasp in South Africa (see Fig. 1 and Table 1). Two trees approximately 100 m apart were sampled per locality resulting in a total of 46 trees. Host trees were identified to species level (*A. longifolia* or *A. floribunda*). Collection sites were classified as “core” (the original release sites) or “edge” (areas into which the wasp is considered to have spread naturally). Fifteen (15) galls were collected from each tree and brought to the laboratory. Following dissection of these galls, adults, pupae and larvae were collected and stored in absolute ethanol at 4° C.

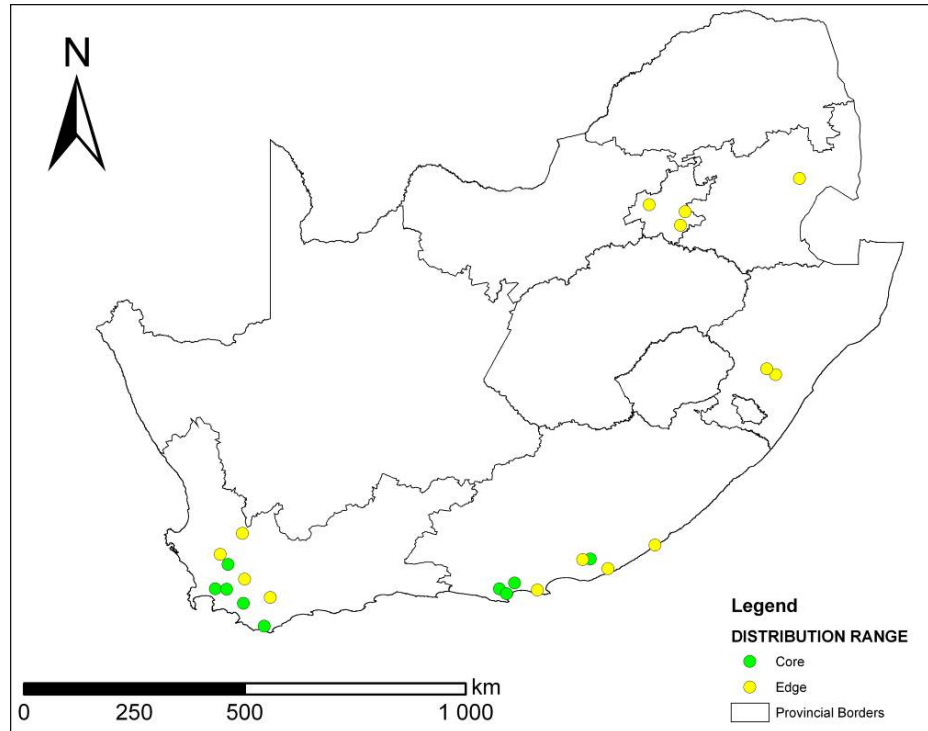


Fig. 1 Map of South Africa indicating the collection localities for *T. acaciaelongifoliae*. Provincial boundaries are indicated. Core sites are indicated in green filled circles and edge sites in yellow filled circles.

2.3.2 Laboratory procedures and sequence treatment

Genomic DNA was extracted from one to four individuals from each locality using the DNeasy Tissue Kit (Quiagen) following the manufacturer's instructions. Whole individual adults, pupae or larvae were used in the DNA extraction. A fragment on the 5' portion of the mitochondrial COI gene was amplified using the insect primers (LCO1490 and HCO2198) as described by Folmer *et al.* (1994). PCR reaction conditions were as follows: an initial denaturation step at 96° C for 5 min. followed by 35 cycles of 96° C for 30 s, annealing at 42° C for 30 s and 72° C for 55 s. A final extension step at 72° C for 1 min. completed the reactions. PCR products were electrophoresed in 1% agarose and gel purified using the DNA purification Kit (GE Healthcare). Sequencing reactions were performed using Big Dye chemistry (version 3; Applied Biosystems, Inc.). Centrisep spin columns (Princeton

Separations) were used to clean sequencing cocktails. The products were analyzed on a 3100 ABI automated sequencer (Applied Biosystems, Inc.). Electropherograms of the raw sequences were checked by eye and edited with Sequence EditorTM software version 1.0.3a (Applied Biosystems, Inc.).

2.3.3 Data analysis

Sequences were aligned using Clustal X (Thompson *et al.* 1997) and verified by eye. Haplotype (h) and nucleotide diversities (π) were calculated using Arlequin 3.1 (Excoffier *et al.* 2005). A haplotype network was constructed using TCS 1.21 (Clement *et al.* 2000). The genetic structure coefficient Φ_{ST} was calculated using Arlequin 3.1 and 1000 permutations were used to test if the Φ_{ST} value was significantly different from random data. Relationships between the observed haplotypes and host plants (*A. longifolia* or *A. floribunda*) as well as between the observed haplotypes and distribution range classification (core or edge) under a null hypothesis of no dependence among variables, were analysed using a one-way Chi-square test implemented in PAST (Palaeontological Statistics, Hammer *et al.* 2001). The chi-square test is designed to determine the relationship between two variables, and the level of significance is assessed by comparing the observed chi-square value with the tabulated value for the given degrees of freedom (Sokal & Rohlf 1995, Zar 1999).

Rarefaction is an interpolation method that estimates the (assumed) total number of species in a given area based on partial sampling of the area (Gotelli & Colwell 2001, Hughes & Hellmann 2005). This method, although typically applied in ecological studies, has recently been used to determine whether haplotype diversity has been sampled to completion and if not, what the expected number of haplotypes would be (Fornia *et al.* 2007, Myburgh *et al.* 2007). To determine whether genetic variation in *T. acaciaelongifoliae* across South Africa was

adequately sampled (Muirhead *et al.* 2008) a sample-based rarefaction curve was generated using 50 randomizations and sampling without replacement in EstimateS 8.0 (Gotelli & Colwell 2001, Colwell 2005). Haplotypes of *T. acaciaelongifoliae* were plotted against the number of localities sampled. Estimators were compared with one another based on their deviations from the observed sample-based rarefaction curves for the localities (see Palmer 1990, Brose *et al.* 2003, Hortal *et al.* 2006).

2.4 Results

2.4.1 Sequence variation

Polymerase chain reaction (PCR) and sequencing resulted in a complete data set of 633 bp of the COI gene for 53 *T. acaciaelongifoliae* specimens. The most frequent haplotype (TH1) was found at all the localities except East London in the Eastern Cape and Elim in the Western Cape. Haplotype TH2 was found in 13 of the 23 localities. The third haplotype (TH3) was found only at the locality of Grahamstown (Eastern Cape Province) (Table 1).

Table 1 Sampling localities (including geographic coordinates) for *T. acaciaelongifoliae*. Sample sizes, core and edge localities are indicated, as well as host plant included per locality. The occurrence of the three mitochondrial DNA haplotypes is indicated.

Locality (Province)	Sample size	Latitude	Longitude	Distribution Range	Host plant	Haplotype		
						1	2	3
Grahamstown (Eastern Cape)	2	33.312	26.532	Core	<i>A. longifolia</i>	TH1	-	TH3
Andrieskraal (Eastern Cape)	2	33.766	24.684	Core	<i>A. longifolia</i>	TH1	TH2	-
Hankey (Eastern Cape)	2	33.828	24.874	Core	<i>A. longifolia</i>	TH1	TH2	-
Humansdorp (Eastern Cape)	3	34.026	24.772	Core	<i>A. longifolia</i>	TH1	TH2	-
Port Alfred (Eastern Cape)	2	33.590	26.890	Edge	<i>A. longifolia</i>	TH1	TH2	-
Salem (Eastern Cape)	2	33.471	26.484	Edge	<i>A. longifolia</i>	TH1	TH2	-
Uitenhage (Eastern Cape)	2	33.750	25.400	Edge	<i>A. longifolia</i>	TH1	TH2	-
East London (Eastern Cape)	4	32.983	27.919	Edge	<i>A. longifolia</i>	-	TH2	-
Kaapsehoop (Mpumalanga)	3	25.552	30.780	Edge	<i>A. longifolia</i>	TH1	-	-
Stellenbosch (Western Cape)	2	33.932	18.859	Core	<i>A. longifolia</i>	TH1	-	-
Caledon (Western Cape)	2	30.183	26.183	Core	<i>A. longifolia</i>	TH1	TH2	-
Demeul (Western Cape)	3	32.798	19.448	Edge	<i>A. longifolia</i>	TH1	-	-
Elim (Western Cape)	2	30.750	26.183	Core	<i>A. longifolia</i>	-	TH2	-
Franschhoek (Western Cape)	2	33.906	19.115	Core	<i>A. longifolia</i>	TH1	-	-
Stormsvlei (Western Cape)	3	34.089	20.088	Edge	<i>A. longifolia</i>	TH1	TH2	-
Tulbagh (Western Cape)	4	33.290	19.139	Core	<i>A. longifolia</i>	TH1	-	-
Worcester (Western Cape)	2	33.645	19.443	Edge	<i>A. longifolia</i>	TH1	-	-
Porterville (Western Cape)	2	26.011	18.993	Edge	<i>A. longifolia</i>	TH1	TH2	-
Krugersdorp (Gauteng)	1	26.095	27.799	Edge	<i>A. longifolia</i>	TH1	-	-
Springs (Gauteng)	3	31.467	28.367	Edge	<i>A. floribunda</i>	TH1	TH2	-
Heidelberg (Gauteng)	1	26.507	28.346	Edge	<i>A. floribunda</i>	TH1	-	-
Hilltown (KwaZulu-Natal)	2	29.441	30.115	Edge	<i>A. longifolia</i>	TH1	-	-
Cedara (KwaZulu-Natal)	2	29.533	30.267	Edge	<i>A. longifolia</i>	TH1	TH2	-

Two variable sites were found resulting in three haplotypes (Table 2). These haplotypes occurred at frequencies 0.64 (34 of 53 specimens) (TH1), 0.34 (18 of 53 specimens) (TH2) and 0.02 (1 of 53 specimens) (TH3). Nucleotide diversity (π) was 0.002 ± 0.001 and haplotype diversity (h) was 0.482 ± 0.045 .

Table 2 Two polymorphic sites (positions 63 and 255) resulted in three haplotypes. The frequencies of haplotypes are indicated (number of specimens are indicated in parenthesis).

Base composition			
Haplotype	Position 63	Position 255	Frequency
TH1	G	C	0.64 (34)
TH2	A	T	0.34 (18)
TH3	G	T	0.02 (1)
Total			1.00 (53)

2.4.2 Genetic structure

The haplotype network constructed for *T. acaciaelongifoliae* is shown in Figure 2. Single mutational differences separate the three haplotypes. No significant geographic structuring of haplotypes was found when considering all populations as a single group; an AMOVA indicated that 90.6% of the genetic variation was accounted for by within-population variation ($\Phi_{ST} = 0.094$, $P = 0.288$). There was also no significant relationship between the geographic occurrence of haplotypes and host plant ($\chi^2 = 2$, $P = 0.157$, $n = 6$) with haplotypes TH1 and TH2 found on both *A. longifolia* and *A. floribunda* (see Table 1). Haplotype TH3 was found only on *A. longifolia* but this may be a sampling artefact rather than a host preference. Likewise, no significant relationship was found between haplotypes and distribution range of the wasp (core vs. edge) ($\chi^2 = 3.333$, $P = 0.068$, $n = 6$).

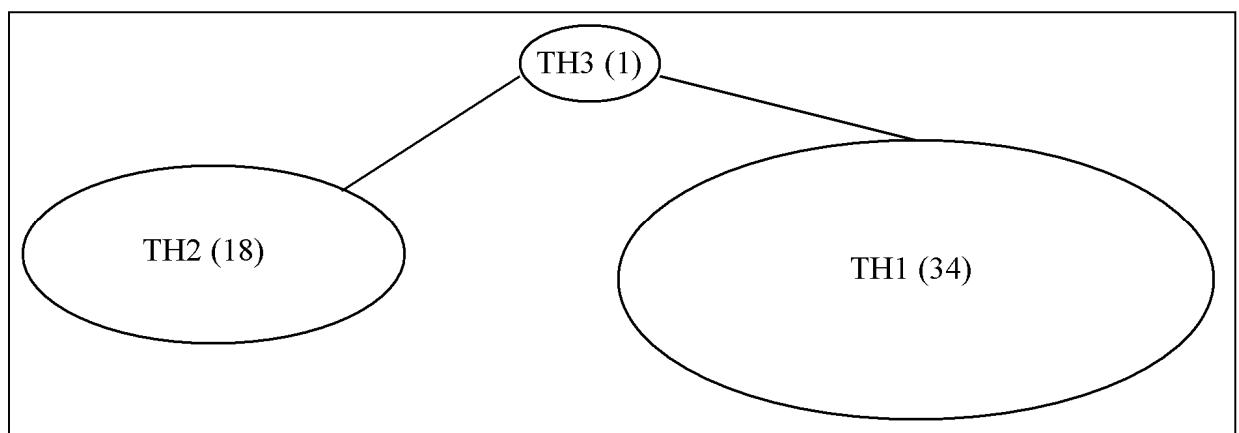


Fig. 2 Statistical parsimony network created in TCS depicting the relationships between haplotypes. Oval sizes are drawn according to the number of individuals (given in brackets).

The calculated rarefaction curve was asymptotic. Both rarefaction curve and the Jack2 estimator indicate that three was the maximum number of haplotypes present across South Africa. It therefore shows that *T. acaciaelongifoliae* haplotypes were sampled to completion for the number of localities (Fig. 3). Jack2 was found to be the best estimator for the localities data.

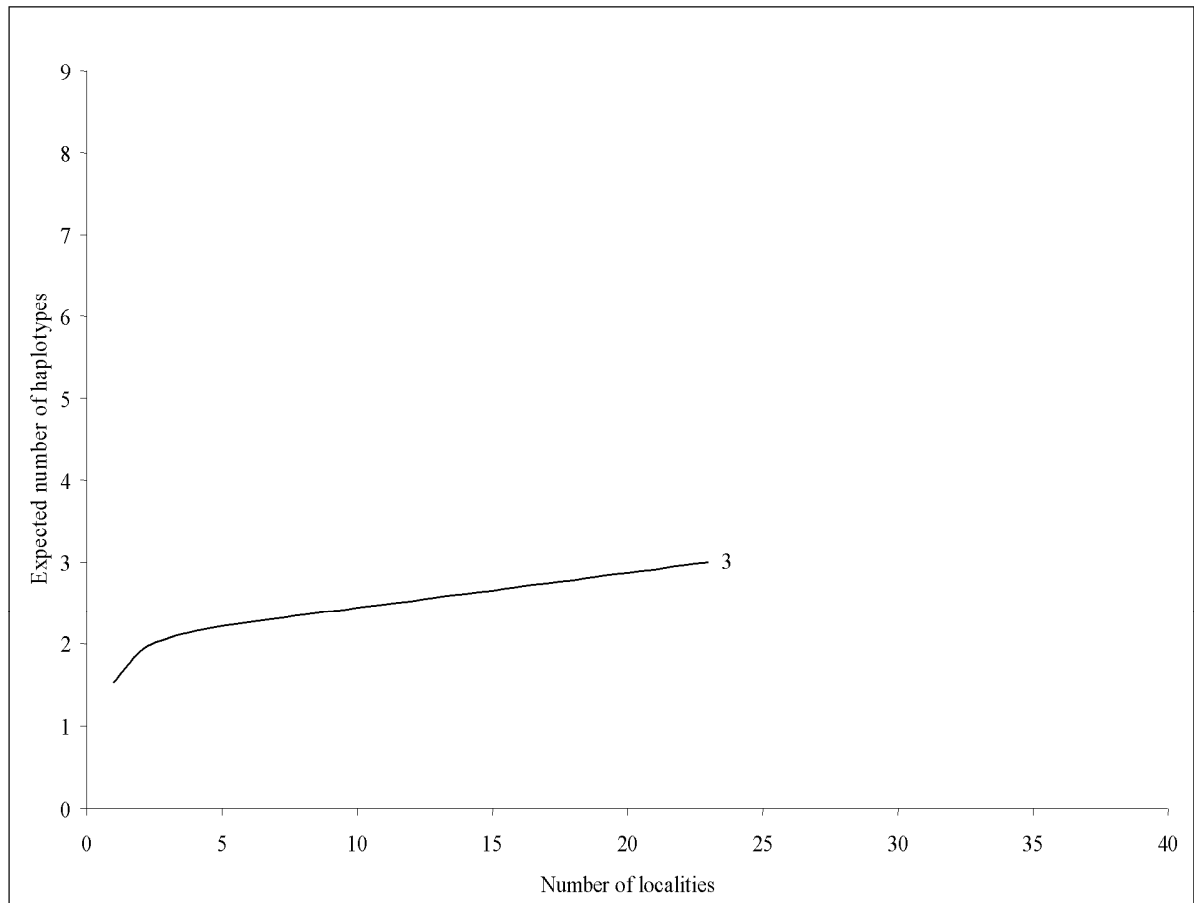


Fig. 3 Rarefaction curve of *T. acaciaelongifoliae* haplotypes in South Africa based on 23 sampling localities. Number in front of the curve refers to the estimated number of haplotypes based on Jack2.

2.5 Discussion

Within South Africa, the bud gall-forming wasp is characterized by low levels of genetic variation. Although this study is based on a limited sample size, it is doubtful whether the inclusion of additional specimens will result in a significant increase in genetic diversity since the rarefaction analysis indicated that all haplotypes have been sampled to completion. Reduced levels of genetic diversity are also in line with previous findings which similarly document reduced levels of mitochondrial DNA variation and lack of genetic structure in introduced species (Lloyd *et al.* 2005, Myburgh *et al.* 2007, Zayed *et al.* 2007, Puillandre *et al.* 2008, but see also Stone *et al.* 2003, Hufbauer *et al.* 2004). Several factors may singly or in

concert account for the low levels of variation and absence of genetic structure in *T. acaciaelongifoliae*. The relative importance of these is discussed below.

Genetic markers differ in their rates of evolution. The choice of a genetic marker that adequately addresses the questions being raised in a study is therefore of utmost importance (Parker & Reichard 1998, Ross 2001, Miura 2007). The mitochondrial COI gene is a marker of choice for insect population level studies (see for example Gunasekera *et al.* 2005, Ball & Armstrong 2006, Scheffer *et al.* 2006, Lee *et al.* 2007) and is suitable for study of genetic structure in situations where reproduction is solely propagated by females as is the case in *T. acaciaelongifoliae* (Noble 1940, Graur 1985). Several studies provide evidence of high levels of mitochondrial DNA variation and structuring in introduced insects. For example, Stone *et al.* (2003) reported mitochondrial DNA structuring in introduced populations of the wasps *A. kollari* and *A. quercuscalicis*. Similarly, Laffin *et al.* (2005) reported structuring in the introduced cabbage seedpod weevil *Ceutorhynchus obstricus*. It is unlikely that the choice of the marker might have contributed substantially to the observed patterns within the wasp in South Africa.

Founder events occur when a subset of a larger population establishes itself in a new environment carrying with it a fraction of the genetic diversity of the parental population. During this process, the founding population passes through a bottleneck further reducing the amount of genetic variation as a result of a reduction in the number of individuals as well as through the effects of genetic drift (Hufbauer & Roderick 2005). This is a well-studied phenomenon with numerous case studies documenting changes in genetic composition and variation as a result of founder events and bottlenecks. For example Stone *et al.* (2003) using allozymes data reported reduced levels of genetic diversity for the introduced wasp *Andricus*

quercuscalicis compared to the co-occurring introduced wasp *A. kollari* and attributed the reasons for the decline in genetic diversity in the former to severe founder effects compared to the latter. Similarly, Baker *et al.* (2003) using microsatellite data reported reduced levels of genetic diversity, fixed alleles and high relatedness in the introduced parasitoid wasp *Diaeretiella rapae* and associated these with a founder effect at the time of introduction. Hinomoto *et al.* (2006) using microsatellite markers found reduced levels of genetic variation in commercial populations of the biocontrol agent poppius *Orius strigicollis* relative to field populations in Japan, and this reduction in diversity was attributed to the effects of a genetic bottleneck and drift.

It is arguable whether a founder effect would have contributed significantly to the observed low levels of genetic variation in the present study. Introductions of the bud gall-forming wasp into South Africa were from different source populations throughout their natural range in Australia thereby (potentially) capturing large proportions of natural diversity (Dennill 1987). In addition, the numbers of introduced individuals (in total, 16257 individuals were released) might have been large enough to avert the negative effects of a bottleneck as the effective population size for *T. acaciaelongifoliae* should not be significantly different to the number of individuals introduced. This species is known to reproduce parthenogenically with a female biased sex ratio (Noble 1940) and displays site fidelity. Although several deaths characterized the first introduction event in 1981, no deaths were reported for the second release event in 1982 (Dennill 1987). There was therefore not a significant decline in the number of individuals that established *per se* from the number originally introduced. This would lend some support to the views that reduced genetic variation is not always a consequence of bottlenecks, and that reduced variation may not necessarily affect the

establishment and successful spread of an introduced species (Hufbauer & Roderick 2005, Miura 2007).

Several authors have argued that mitochondrial DNA may not be selectively neutral (see Ballard & Rand 2005, Hurst & Jiggins 2005, Behura 2006). If true, this would imply that selection could favour specific mitochondrial haplotypes causing those haplotypes to become abundant whilst other, less optimal haplotypes, disappear from a population resulting in a loss of genetic diversity. Following the work of Bazin *et al.* (2006), selection seems to be more prevalent among invertebrates compared to vertebrates. These authors documented the number of adaptive amino acid substitutions compared to those that are non-adaptive (dN/dS ratio) for 13 mitochondrial DNA genes and reported significant differences between vertebrates (average 0.086) and invertebrates (average 0.151) which they attributed to recurrent selective sweeps. In addition, Confalónieri *et al.* (1998) documented mitochondrial DNA variation in the grasshopper *Trimerotropis pallidipennis* along an altitudinal cline. They found no clinal variation (isolation-by-distance) with no correlation between haplotype and geographic sampling locality and attributed this lack of genetic structure along the geographic cline to selection acting along the cline. Therefore selection may have acted on the introduced populations of *T. acaciaelongifoliae* in South Africa resulting in the observed level of genetic variation.

When considering haplodiploid systems (also characterizing *T. acaciaelongifoliae*), much of the neutral variation at nucleotide sites targeted by selection will be rapidly lost in haploid males (because of a lower N_e) leading to a decrease in overall genetic diversity (Maynard Smith & Haigh 1974, Nielsen 2001, Packer & Owen 2001). In addition, should selection favour specific mitochondrial haplotypes, there will be a rapid increase in the

frequency of these haplotypes having the selective advantage, such that some alleles or haplotypes in introduced population will be found in large frequencies relative to others. In the present study, two haplotypes (TH1 and TH2) occurred at high frequency with TH3 found only at a single locality which corresponds to a site of original introduction.

Interestingly, the present study detected no difference in mitochondrial variation or haplotype frequencies when considering two host species (*A. longifolia* and *A. floribunda*) nor when considering core vs. edge sites in the distribution. Previous work have shown co-adaptation among host and parasite haplotypes such that host-specific races of parasites arise through selection on parasite haplotypes to more accurately match their hosts' genetic profiles (see Hufbauer & Roderick 2005, Goolsby *et al.* 2006a & b). This does not appear to be the case with the bud gall-forming wasp in South Africa. Although speculative, this may be the result of environmental selection for specific haplotypes outweighing the formation of haplotypes driven by host specificity. Alternatively, the haplotypes characterizing *T. acaciaelongifoliae* specimens in South Africa may have arisen prior to the divergence of *A. floribunda* from *A. longifolia*, suggesting that the ability to parasitize both hosts evolved prior to the diversification of the hosts' mitochondrial DNA. This was similarly argued for *Diaeretiella rapae*, a parasitoid of aphids introduced into Northern America (see Baer *et al.* 2004). Although these findings would suggest that selection is playing a role in shaping the variation in *T. acaciaelongifoliae* in South Africa, the finding from this study can not be taken as conclusive and further studies would be needed to confirm or refute this.

The time since introduction plays an important role in the amount of genetic variation present in species. Mutations would accumulate over time (this would be proportional to generation time rather than real time), and if the introduction occurred relatively recent, there

might not have been a sufficient number of generations in which to have accumulated genetic variation (in the form of new mutations) (Roderick & Navajas 2003, Crispo & Hendry 2005). The wasp introductions into South Africa occurred ~26 years ago (1981 and 1982) and with a generation time of between 6 months to 1 year (see Noble 1940, Dennill 1987), this would leave between 26 – 52 generations in which mutations could accumulate. Also, given that mutations accumulate at only ~ 2.3% per million years for the COI gene (Brower 1994, Knowles 2000), 26 years would not be enough to accumulate a noticeable amount of genetic variation. However, rapid evolution has been shown to occur over short time scales and few generations (Carroll *et al.* 2007). For example, Gilchrist *et al.* (2001) reported changes in the wing size of the fruit fly *Drosophila subobscura* with latitude two decades after its introduction into the new world. Similarly, rapid evolution for increased growth and survival rates, and development of a cline in flowering phenology has been observed in populations of *Hypericum canariense*, a perennial shrub introduced to Hawaii and California less than fifty years ago (Dlugosh & Parker 2008).

Human-mediated dispersal may add to low genetic variation and homogenization of populations (Therriault *et al.* 2005). Given the low agility of the bud gall-forming wasp (Noble 1940) in combination with the lack of diversification across the entire range of several thousand kilometres in South Africa, I would suggest that human mediated dispersal is partly responsible for generating the observed levels of genetic variation within the wasp (see also Dennill 1987, Hufbauer 2002, Therriault *et al.* 2005).

Although it is widely believed that propagule pressure plays an important role in the successful establishment and spread of an introduced species (Allendorf & Lundquist 2003, Lockwood *et al.* 2005, Miura 2007), this study adds to the growing body of literature that

suggest that propagule pressure may not be of vital importance in some species becoming invasive (or even establishing) (see Grevstad 1999). Memmott *et al.* (2005) showed that invasion success was associated with the first introduction event and not subsequent events of introduction in the biological control psyllid *Arytainilla spartiophila*. Likewise, Hänfling *et al.* (2002) demonstrated that invasive populations of the Chinese mitten crab *Eriocheir sinensis* in America were founded by a single introduction event. Similarly, Myburgh *et al.* (2007) reported low COI sequence variation in invasive populations of springtails on Marion Island and associated that with small propagule size at the time of introduction. Furthermore, Zayed *et al.* (2007) reported establishment of large populations becoming invasive from an initial introduction of few individuals. Although two introductions of wasps into South Africa occurred, only the second introduction of *T. acaciaelongioliae* is considered to have been successful (Dennill 1987).

The indications that some of the well known factors (e.g. large population sizes and propagule pressure) associated with successful establishment and spread of an introduced species in a novel environment may not be a prerequisite for the successful establishment and spread of introduced species, raises an important question; how can a species such as the biocontrol agent *T. acaciaelongifoliae* establish successfully, reach large population sizes and spread across the range of its host despite low genetic diversity? One possible explanation may be that the levels of genetic diversity in the host are matched by the levels of variation in the biocontrol agent. One of the main reasons postulated for the failure to successfully control *Lantana camara*, a highly successful invasive weed, has been the fact that this host species has very high levels of genetic and phenotypic variation with no single biocontrol agent that can match these levels of variation. Although speculative since no data are available for the long-leaved wattle in South Africa, it may be that the levels of variation between host and parasitoid

are equally matched. Given that *T. acaciaelongifoliae* in South Africa is characterized by very low levels of mitochondrial DNA variation, it may be that the genotypes present perfectly match host plant genotype.

In conclusion, *T. acaciaelongifoliae* across South Africa does not seem to suffer any negative effects in spite of low genetic variation. This is because the species have successfully spread across most of South Africa, is characterized by high population sizes, and suffer low rates of parasitism. The species is arguably having an impact on host plant reproduction (Dennill 1987, Dennill & Gordon 1990, Manongi & Hoffman 1995, McGeoch & Wossler 2000). It is very difficult to single out one factor responsible for the low levels of genetic variation in *T. acaciaelongifoliae*, and several factors may have contributed to the low levels of genetic variation observed in *T. acaciaelongifoliae* populations in South Africa. This study adds to a growing body of literature which would question the traditional view about the role of propagule pressure in the establishment and successful spread of introduced species.

Chapter 3: Distribution of genetic variation in Argentine ants across South Africa

3.1 Introduction

The Argentine ant, a species native to South America, has established and become invasive on most continents (Sugiyama 2000, Suarez *et al.* 2001) where it significantly impacts local economies and biodiversity (Christian 2001, Olson 2006, Silverman & Brightwell 2008). It would appear that the spread and success of this ant is largely human-mediated through repeated introductions (Skaife 1953, Markin 1970, Ingram & Gordon 2003, Hirata *et al.* 2008). In both introduced and native range, Argentine ants form colonies or supercolonies (Tsutsui *et al.* 2000, Giraud *et al.* 2002, Pedersen *et al.* 2006). A colony refers to many interconnected nests that are characterized by the presence of several queens and lack of intracolony or internest aggression (Markin 1970, Tsutsui *et al.* 2000). A supercolony denotes several interconnected nest of multi-queen colonies spread over large geographic area with no aggression between mates (Jaquiéry *et al.* 2005, Corin *et al.* 2007a).

Throughout the introduced range of the Argentine ant, colony number and size varies greatly. For example, a single colony has been reported from Chile and New Zealand (Dreier *et al.* 2005, Corin *et al.* 2007a) compared with more than six colonies throughout the USA (Buczkowski *et al.* 2004, Thomas *et al.* 2006). One of the smallest colonies reported to date stretches across less than 1 km in the USA (Holway & Suarez 2004) whereas a single colony is distributed across more than 6000 km in Europe (Jaquiéry *et al.* 2005, Wetterer & Wetterer 2006). There is also a noticeable difference in the amount of genetic variation reported for invasive colonies with the majority of colonies displaying reduced levels of variation compared to colonies in their native range (Tsutsui *et al.* 2000, Giraud *et al.* 2002).

Several factors may account for the reduced level of genetic diversity which largely facilitates the formation of super colonies. Amongst these are bottlenecks during introduction, selection for specific genotypes and/ or allelic cleansing. However, empirical evidence implicating these evolutionary processes is scanty (Buczkowski *et al.* 2004). Tsutsui *et al.* (2000) proposed that bottlenecks reducing genetic variation facilitated the formation of a large supercolony in California. In contrast, Giraud *et al.* (2002) argued that Argentine ant colonies in southern Europe did not experience a major genetic bottleneck since the populations or colonies they studied were comparatively genetically diverse. Instead, these authors attributed the formation of the supercolony in southern Europe to selection against (or cleansing of) alleles coding for recognition cues. Importantly, the specific alleles coding for the recognition cues in ants have not been identified and this hypothesis therefore still needs to be verified.

Various levels of genetic structuring have been reported across the introduced range of the Argentine ant. Colonies from California and the south-eastern USA tend to be characterized by little to moderate genetic structuring (Buczkowski *et al.* 2004) whereas colonies from southern Europe and Japan show higher levels of geographic structuring (Jaquiéry *et al.* 2005, Hirata *et al.* 2008). Geographic partitioning of genetic variation has been linked to aggression displayed between ant colonies (Thomas *et al.* 2007) and these two characteristics (behavioural aggression and genetic distinctness) are frequently used to delimit colony boundaries (Tsutsui *et al.* 2000, Buczkowski *et al.* 2004, Jaquiéry *et al.* 2005, Hirata *et al.* 2008).

The Argentine ant was accidentally introduced into South Africa along with animal fodder and was first recorded in Stellenbosch around 1908 (Skaife 1953, Prins 1978). The occurrence of Argentine ants in South Africa has been documented mainly through ecological studies (Christian 2001, Wild 2004). Few investigations have documented population genetic

profiles of Argentine ants in South Africa. To date, only a single study included samples from South Africa (Tsutsui *et al.* 2001) with the findings from this study being somewhat restricted in that ants from only three sites in the Western Cape Province were included. Notwithstanding limited geographic coverage, Tsutsui *et al.* (2001) suggested that more than one introduction event of the Argentine ant into South Africa may have taken place based on large genetic differences between ant populations. Specifically, ant populations from Caledon were significantly different to those from Cape Point and Betty's Bay. A cluster analysis indicated that ants from South Africa clustered with ants from Rosario, Otamendi and Buenos Aires (Argentina) indicating the possibility of multiple introductions into South Africa.

3.2 Research questions

To address the lack of genetic information about the Argentine ant in South Africa, five questions were posed for this study:

- What are the levels of genetic variation characterizing the Argentine ant in South Africa?
- Are Argentine ant populations in South Africa genetically structured?
- If the Argentine ant is indeed genetically structured, might these represent more than one supercolony?
- Can the source population(s) for the introductions into South Africa be identified?
- Is there evidence for demographic and evolutionary processes such as genetic bottlenecks or natural selection as well as population expansion in shaping the distribution of genetic diversity in Argentine ants across South Africa?

3.3 Material and methods

3.3.1 Sample collection and DNA extraction

A total of 314 ant workers were collected from 35 geographic localities throughout South Africa (Fig. 4). Ants were brought back to the laboratory at Stellenbosch University and stored in absolute ethanol at 4° C. Total genomic DNA was extracted from worker ants using a commercial DNA extraction kit (Qiagen).

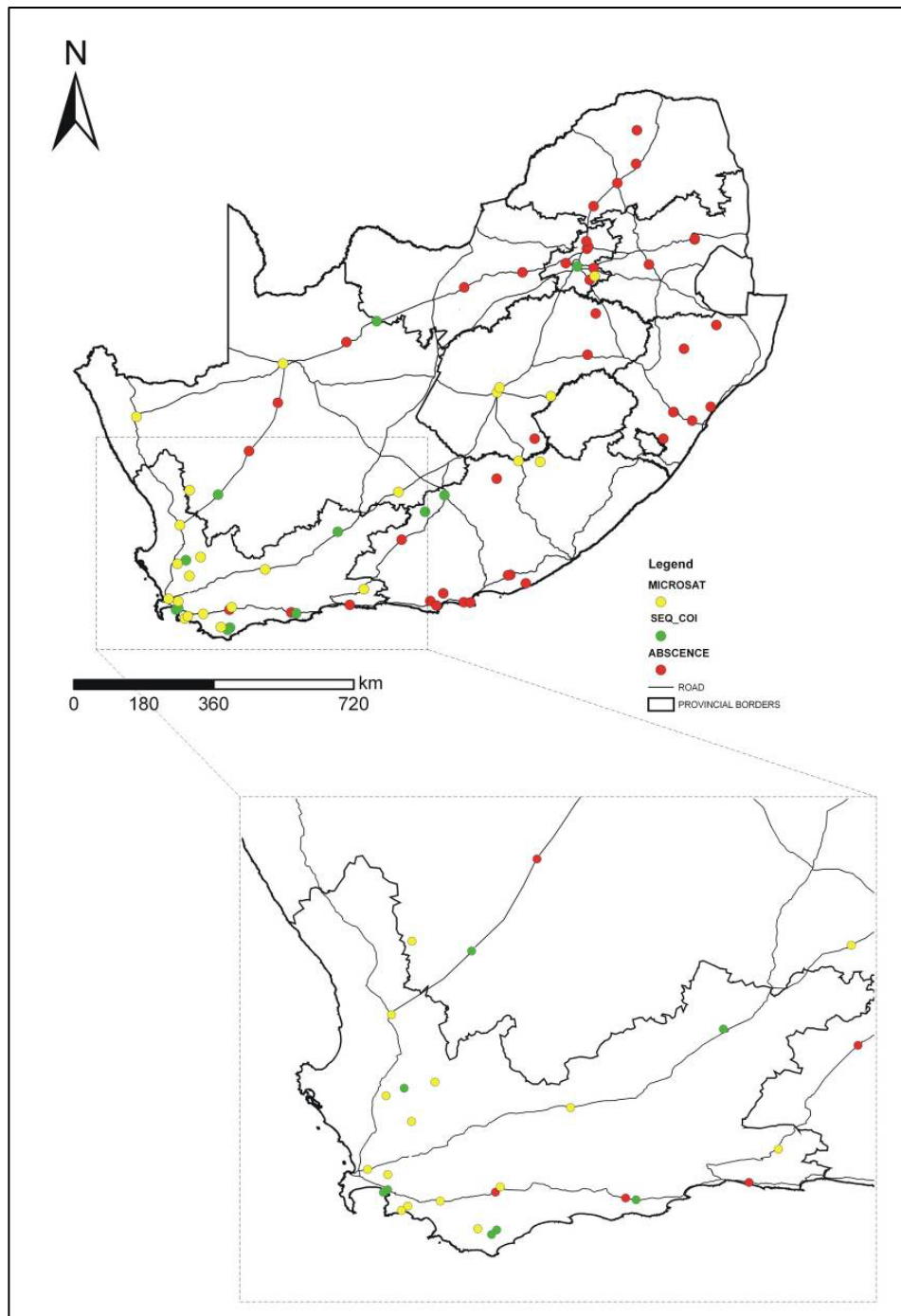


Fig. 4 Map of South Africa indicating the sampling locations for the Argentine ant, *L. humile*. The main road network in South Africa is indicated. Red circles indicate sites that were visited but where Argentine ants were not found. Localities where Argentine ants were present and for which mitochondrial sequence data are available are indicated in green. Localities where Argentine ants were present and for which both mitochondrial and microsatellite data are available are indicated in yellow.

3.3.2 Mitochondrial DNA data analysis

3.3.2.1 Laboratory procedures and sequence treatment

To assess genetic variation across South Africa, a portion of the mitochondrial COI gene was targeted. For this, sequences were generated for 101 ants from 35 localities using the Folmer *et al.* (1994) insect primers LCO1490 and HCO2198 (see Fig. 4 and Table 3). PCR profiles included an initial denaturation step of 5 min. at 94° C followed by 30 cycles of 94° C for 30 s, annealing at 42° C for 30 s and extension at 72° C for 45 s. A final extension for 7 min. at 72° C completed the reactions.

Comparative cytochrome b data for non-South African populations were available. To determine possible source population for introduction into South Africa, cytochrome b data were generated for a subset of ants included in the COI analyses (11 workers from 11 localities) using the primer pair CB1 and CB2 (Chiotis *et al.* 2000). These localities (Elim2, Porterville2, Bloemfontein2, Nigel, Springbok, Lady Brand, Lady Grey, Lady Brand, Caledon, Betty's Bay and Kogelberg Nature Reserve; see Table 3) were selected to provide good geographic coverage with ant specimens chosen to be representative of the COI and microsatellite variation. PCR parameters were identical to those used for the COI gene.

Amplicons were electrophoresed in 1% agarose. Bands were excised and purified using a commercial DNA purification Kit (GE Healthcare). Sequencing reactions were performed using Big Dye chemistry (version 3; Applied Biosystems, Inc.). Centrisep spin columns (Princeton Separations) were used to clean sequencing cocktails. The products were analyzed on an ABI 3100 automated sequencer (Applied Biosystems, Inc.). Electropherograms of the raw sequences were checked by eye and edited with Sequence EditorTM software version 1.0.3a (Applied Biosystems, Inc.). All sequences (COI and cytochrome b) were manually edited and aligned using the Clustal X algorithm (Thompson *et al.* 1997).

Table 3 List of localities, geographic positions (latitude and longitude in decimal degrees, altitude in metres), composition of the observed COI and cytochrome b haplotypes as well as localities (= populations) for which microsatellite data were included.

Locality	Lat	Long	Alt (m)	COI haplotypes					Cyt b hap	Microsat
				1	2	3	4	5		
Stellenbosch	33.932	33.863	127	-	LH2	-	LH4	LH5	x	Yes
Somerset West	34.078	18.844	53	-	-	-	-	LH5	x	No
Strand	34.100	18.850	136	-	-	-	-	LH5	x	No
Tulbagh	33.349	19.111	150	-	-	-	-	LH5	x	Yes
Porterville1	32.984	19.030	314	-	-	-	-	LH5	x	No
Porterville2	33.067	18.832	28	LH1	-	-	-	-	SA6	Yes
De Meul	32.916	19.368	998	-	-	-	-	LH5	x	Yes
Stormsvlei	34.070	20.085	134	-	-	-	-	LH5	x	Yes
Elim1	34.592	19.990	23	-	-	-	-	LH5	x	No
Elim2	34.530	19.839	164	LH1	-	LH3	-	-	SA5	Yes
Bredasdorp	34.542	20.046	56	-	-	-	-	LH5	x	No
Laingsburg	33.197	20.857	1141	-	-	-	-	LH5	x	Yes
Uniondale	33.654	23.138	721	-	-	-	-	LH5	x	Yes
Albertina	34.212	21.575	207	-	-	-	-	LH5	x	No
Bellville	33.876	18.629	104	-	-	-	-	LH5	x	Yes
Beaufort West	32.333	22.533	955	-	-	-	-	LH5	x	No
Betty's Bay	34.360	18.960	9	-	-	-	-	LH5	SA4	Yes
Caledon	34.225	19.427	289	-	-	-	-	LH5	SA4	Yes
KogelbergR	34.322	18.966	32	-	-	-	-	LH5	SA7	Yes
Clanwilliam	32.176	18.891	86	-	-	-	-	LH5	x	Yes
Middleburg	31.485	25.001	1240	-	-	-	-	LH5	x	No
Nieu Bethesda	31.867	24.554	1316	-	-	-	-	LH5	x	No
Lady Grey	30.713	27.210	1646	-	-	-	-	LH5	SA7	Yes
Aliwal North	30.693	26.710	1336	-	-	-	-	LH5	x	Yes
Lady Brand	29.1977	27.458	1600	-	-	-	-	LH5	SA7	Yes
Bloemfon1	28.998	26.274	450	-	-	-	-	LH5	x	Yes
Bloemfon2	29.110	26.215	1363	LH1	-	-	-	-	SA6	Yes
Richmond	31.413	23.937	799	-	-	-	-	LH5	x	Yes
Calvinia	31.476	19.770	975	-	-	-	-	LH5	x	No
Kuruman	27.464	23.437	1317	-	-	-	-	LH5	x	No
Springbok	29.673	17.884	920	-	-	-	-	LH5	SA7	Yes
Niewoudtville	31.369	19.116	779	-	-	-	-	LH5	SA7	Yes
Upington	28.443	21.271	848	-	-	-	-	LH5	x	Yes
Nigel	26.422	28.471	1552	-	-	-	-	LH5	SA5	Yes
Greenside	26.200	28.067	1000	-	-	-	-	LH5	x	No

3.3.2.2 Genetic diversity

Standard measures of genetic diversity such as nucleotide (π) and haplotype (h) diversities were estimated using Arlequin 3.1 (Excoffier *et al.* 2005). Uncorrected (p) sequence divergences separating specimens were calculated in PAUP*4.0b10 (Swofford 2003). A haplotype network, indicating the evolutionary relationships among the COI haplotypes, was constructed in TCS (Clement *et al.* 2000).

To determine whether genetic variation in Argentine ants across South Africa was adequately sampled (see Forna *et al.* 2007, Muirhead *et al.* 2008), a sample-based rarefaction curve was generated using 50 randomizations and sampling without replacement in EstimateS 8.0. COI haplotypes were plotted against the number of localities sampled. Estimators were compared with one another based on their deviations from the observed sample-based rarefaction curves for localities. The best estimator for the COI data was Jack1 (following Palmer 1990, Brose *et al.* 2003, Hortal *et al.* 2006). Like Jack2, Jack1 is a nonparametric estimator and it is based on number of species (rare species) occurring in one sample (Colwell 2005).

3.3.2.3 Genetic differentiation across South Africa based on COI sequence data

To determine whether genetic variation in Argentine ant populations across South Africa is geographically structured, two approaches were followed. First, Φ_{ST} as implemented in Arlequin 3.1 was calculated. For this, all sampling localities were considered as a single group. Significance was determined through 1000 permutations. Second, a non-metric multidimensional scaling (NMDS) method, an ordination that groups similar populations together, was performed using PAST software (Hammer *et al.* 2001) and the resulting figure was edited in CorelDraw 12 (Corel Corporation 2003). The algorithm of NMDS implemented

in PAST uses random initial conditions, one of which is the principal coordinate (PCO) with 11 iterations for each run (Hammer *et al.* 2001). The NMDS estimates a spatial representation for a given dissimilarity matrix whereby the rank order of the distances between the embedded objects agrees with the rank order of the dissimilarities in as much as possible (Kruskal 1964). The NMDS does this by minimizing a cost function called stress (Kruskal 1964). Stress value measures how the configuration matches the original data and it ranges from zero (perfect match) to one (total mismatch).

3.3.2.4 Colony structure and composition

Given the presence of significant genetic structuring (see results section) the possible presence of multiple colonies in South Africa was investigated. A one-way analysis of similarity (ANOSIM; Clarke & Green 1988) implemented in PAST was used to estimate the level of differentiation between groups (possibly colonies) within South Africa. Significance for the coefficient of similarity R was obtained through 10000 permutations. ANOSIM compares within and among group differences such that when two groups are identical or very similar, the “within group” difference would be small relative to the “between groups” value. The reported R value is an estimate of degree of similarity. Similarity coefficient R ranges from -1 to 1 where large positive R values signifies dissimilarity between groups, 0 means lack of pattern (random) and values below zero denote similarity.

The absence of gene flow between groups would provide further evidence to support distinct colonies (Jaquière *et al.* 2005, Thomas *et al.* 2006). Gene flow was determined between groups (possibly colonies) using the Hudson *et al.* (1992) model implemented in DnaSP 4 (Rozas *et al.* 2003).

3.3.2.5 Putative source population(s) for Argentine ants in South Africa based on cytochrome b data.

Cytochrome b sequence data were generated for Argentine ants representing their distribution range in South Africa and compared to those from across the world (data were kindly donated by Andrew Suarez, University of Illinois, and Neil Tsutsui, University of California, Berkeley). The final dataset represented haplotypes from ten countries (Argentina, Brazil, Australia, New Zealand, South Africa, Italy, USA, Chile, Bermuda and Hawaii; the latter two are considered separately from the USA based on geographic location). All specimens included as well as geographic sampling localities are provided in Table 4.

Putative source population(s) was investigated following Corin *et al.* (2007a). A neighbour joining tree was constructed from HKY + G (0.211) distances because it is the optimal model selected by Modeltest (Posada & Crandal 2001, Miller III & Crespi 2003). A haplotype network clustering the South African haplotypes with those from across the world was constructed in TCS 1.21 to depict evolutionary relationship among haplotypes.

Table 4 Argentine ants were included from across the world in an attempt to identify putative source populations for introduction into South Africa. Haplotypes are based on 407 bp of cytochrome b data. The South African localities are shown in bold. The South African haplotypes indicated here are based on 738 bp of sequence data

Country	Locality	Haplotype	Data Source
Argentina	Ocampo	OCA1	Andrew Suarez & Neil Tsutsui
Argentina	Ocampo	Oca010	Andrew Suarez & Neil Tsutsui
Argentina	Ocampo	OC11	Andrew Suarez & Neil Tsutsui
Argentina	Rosario	RO	Andrew Suarez & Neil Tsutsui
Argentina	Costanera Sur	CS2	Andrew Suarez & Neil Tsutsui
Argentina	Ibicuy	CUY	Andrew Suarez & Neil Tsutsui
Argentina	Pre delta	PRE1	Andrew Suarez & Neil Tsutsui
Argentina	Alvear	AV	Andrew Suarez & Neil Tsutsui
Argentina	Ibate	IT	Andrew Suarez & Neil Tsutsui
Argentina	Colon	CO	Andrew Suarez & Neil Tsutsui
Brazil	Passo do Lontra	PL1	Andrew Suarez & Neil Tsutsui
Brazil	Passo do Lontra	PL2	Andrew Suarez & Neil Tsutsui
Brazil	Serra do Jappi	SJ1	Andrew Suarez & Neil Tsutsui
Brazil	Serra do Jappi	SJ2	Andrew Suarez & Neil Tsutsui
Brazil	Monte Verde	MV	Andrew Suarez & Neil Tsutsui
Australia	Perth	Perth	Andrew Suarez & Neil Tsutsui
Australia	Melbourne	Crozier	Andrew Suarez & Neil Tsutsui
New Zealand	•	EF	Andrew Suarez & Neil Tsutsui
South Africa	Cape Point	SA1	Andrew Suarez & Neil Tsutsui
South Africa	Betty's Bay1	SA2	Andrew Suarez & Neil Tsutsui
South Africa	Caledon1	SA3	Andrew Suarez & Neil Tsutsui
South Africa	Caledon	SA4	Present study
South Africa	Betty's Bay	SA4	Present study
South Africa	Elim2	SA5	Present study
South Africa	Nigel	SA5	Present study
South Africa	Porterville2	SA6	Present study
South Africa	Bloemfontein2	SA6	Present study
South Africa	Springbok	SA7	Present study
South Africa	Kogelberg Reserve	SA7	Present study
South Africa	Lady Grey	SA7	Present study
South Africa	Lady Brand	SA7	Present study
South Africa	Nieuwoudtville	SA7	Present study
Italy	Rome	RIW	Andrew Suarez & Neil Tsutsui
USA	Sweet Water, California	SW2	Andrew Suarez & Neil Tsutsui
USA	Sweet Water, California	SW3	Andrew Suarez & Neil Tsutsui
USA	New Orleans, Luisiana	NOr1	Andrew Suarez & Neil Tsutsui
USA	Los Angeles, California	LA8	Andrew Suarez & Neil Tsutsui
USA	Lake Skinner, California	LS	Andrew Suarez & Neil Tsutsui
USA	California	UCSD	Andrew Suarez & Neil Tsutsui
Chile	Vina del Mar	chis	Andrew Suarez & Neil Tsutsui
Bermuda	Bermuda	•	Andrew Suarez & Neil Tsutsui
Hawaii	Kilauea	H11	Andrew Suarez & Neil Tsutsui
Hawaii	Kilauea	H12	Andrew Suarez & Neil Tsutsui

• Not available

3.3.3 Microsatellite data analysis

3.3.3.1 Laboratory procedures and data treatment

For the microsatellite analyses, 10 worker ants were included per population (= sampling locality) (see Table 3 & Fig. 4). In total, 230 specimens from 23 localities were analysed for six polymorphic microsatellite loci (Lhum11, Lhum13, Lhum28, Lhum35, Lhum39 and Lhum52; Krieger & Keller 1999). PCR reactions followed the methodology described in Krieger & Keller (1999) with optimization in the laboratory. PCR cycles included an initial denaturation at 96° C for 5 min. followed by 35 cycles of 96° C for 30 s, specific primer annealing for 45 s and 72° C for 30 s. A final extension for 2 min. at 72° C completed all reactions. Microsatellite reactions were analysed on an ABI 3100 automated sequencer (Applied Biosystems, Inc.). Allele sizes were scored in Genemapper Software 3.7 using standard allelic sizes (Applied Biosystems, Inc.).

3.3.3.2 Genetic diversity

Standard measures of genetic diversity (mean number of alleles (A_O), observed (H_O) and expected (H_E) heterozygosities) were estimated for each population across all loci. Hardy-Weinberg equilibrium tests (HWE) were performed for each locus and population individually as well as across all loci and populations to determine whether the loci and populations are in equilibrium.

To determine whether genetic variation was adequately sampled (Muirhead *et al.* 2008), a sample-based rarefaction curve was generated using 50 randomizations and sampling without replacement in EstimateS 8.0. Alleles were plotted against the number of localities sampled (Leberg 2002, Kalinowski 2004, Belkhir *et al.* 2006). Estimators were compared with one another based on their deviations from the observed sample-based rarefaction curves for

the localities. Jack1 was the best estimator for the data (see Palmer 1990, Brose *et al.* 2003, Hortal *et al.* 2006).

Although deviations from HWE may be caused by factors such as genetic drift and selection, genotyping artefacts such as null alleles have also been shown to affect equilibrium and cause heterozygosity deficits. Notwithstanding, very few studies to date have tested for the presence of null alleles (but see Ingram & Gordon 2003, Buczkowski *et al.* 2004, Jaquiéry *et al.* 2005, Hirata *et al.* 2008). In the present study, the presence of null alleles was assessed using MICROCHECKER 2.1.3 (van Oosterhout *et al.* 2004). Adjusted allele frequencies based on Brookfield (1996) was compared to the observed allele frequencies using the chi square test in STATISTICA 7.0 (Stat soft).

3.3.3.3 Genetic differentiation

To determine how genetic variation, based on microsatellite data, is spatially distributed, several approaches were followed. First, to determine whether variation is significantly structured across the landscape, R_{ST} was calculated in Arlequin 3.1 considering all populations as a single group. Significance was determined through 1000 permutations of the data. Second, a pattern of isolation-by-distance occurs when gene flow is significantly correlated with geographic distances among populations. To test whether such a correlation exist across South Africa, a Mantel test was implemented in SPAGeDi 1.2 (Hardy & Vekermans 2002). Significance for the relationship between genetic and geographic distances was tested through 1000 permutations. Third, similar to the mitochondrial DNA analyses, NMDS was performed using PAST and the resulting figure was edited in CorelDraw 12 (Corel Corporation 2003).

3.3.3.4 Colony structure and composition

To investigate the possible presence of multiple colonies in South Africa, several approaches were followed. First, the presence of private alleles might suggest distinct populations with limited gene flow. CONVERT (Glaubitz 2004) was used to identify populations with high numbers of private alleles. Secondly, ANOSIM as implemented in PAST was performed with significance for R determined through 10000 permutations. Third, the number of migrants (gene flow) between groups was determined using the private allele method of Slatkin (1985) as implemented in GENEPOP (Raymond & Rousset 1995) with all populations treated as a single cluster (colony). Gene flow was also calculated between putative colonies (based on the results of this study) using GENEPOP.

Relatedness (overall) was estimated considering all populations as a single colony as well as for putative colonies (populations within a colony would be more related than across colonies). For this, the program RELATEDNESS 5.0 (Queller & Goodnight 1989) was used, with relatedness coefficient r weighted by individuals and standard errors obtained by jack-knifing over populations. Significance for the observed r value (considering all populations as a single colony as well as within the two groups) was obtained using a one sample t-test implemented in PAST.

3.3.3.5 Demographic and evolutionary processes

3.3.3.5.1 Bottleneck

It was argued by Tsutsui *et al.* (2000) that a reduction in population size (bottleneck) during an introduction would lead to reduced levels of genetic variation across the introduced range thereby facilitating the formation of large super colonies. To test whether Argentine ants suffered a bottleneck during their introduction into South Africa, heterozygosity excess was

determined across all loci for each population using the two phase mutation model (TPM) (Di Rienzo *et al.* 1994) implemented in BOTTLENECK 1.2 (Cornuet & Luikart 1996). Under this approach, populations that have undergone a genetic bottleneck will show increased levels of heterozygosity compared to that of a population in mutation-drift equilibrium given the same number of alleles. Significance for heterozygosity excess was determined using the Wilcoxon sign-rank test (Cornuet & Luikart 1996, Piry *et al.* 1999).

3.3.3.5.2 Natural selection and population expansion

Spatial autocorrelation measures the degree of similarity between pairs of alleles at a given distance; the null hypothesis being no spatial autocorrelation (Sokal & Oden 1978a & b, Reusch *et al.* 1999). The spatial autocorrelation coefficient used here is Moran's I (Moran 1950) and it was calculated as pairwise relationship coefficient (kinship coefficient) across all localities (18 distance intervals) included in the microsatellite analyses. Spatial autocorrelation analysis was implemented in SPAGeDi 1.2 (Hardy & Vekermans 2002). Moran's I coefficient varies from -1 to +1 depending on the magnitude and direction of the correlation. Several predictions can be tested. First, for a population under selection, a plot of Moran's I coefficients against linear distance will reveal a genetic cline for a locus or some loci but not for all loci in a study (Reusch *et al.* 1999, Stone *et al.* 2003). Under this prediction, Moran's I values for certain loci at small spatial scale are expected to decline from highly significant positive values to highly significant negative values at large spatial scale. Secondly, for a population expanding its range, a plot of Moran's I coefficient against distance will show a genetic cline for the whole genome (all loci will be affected) (Long & Singh 1995, Kennington *et al.* 2003, Stone *et al.* 2003). Under this pattern, Moran's I coefficients for all loci studied are expected to drop from significantly positive and high values at small distances to highly significantly negative at large distances. Thirdly, for populations under the influence of

isolation-by-distance and thus affected by genetic drift and short range gene flow, a plot of Moran's I values against distance will indicate the presence of fine scale genetic structure (McFadden & Aydin 1996, Kennington *et al.* 2003, Fredsted *et al.* 2005). Here Moran's I values are expected to decrease from highly significant values at small distances to non-significant at large distances. Results from this analysis were considered in combination with the Mantel test results which tests for isolation-by-distance across the entire geographic range of Argentine ants in South Africa. Significance tests for the Morans' I values were determined using 10000 permutations.

3.4 Results

3.4.1 Mitochondrial DNA

3.4.1.1 Genetic diversity

For the mitochondrial COI gene, 618 bp was generated for 101 specimens from 35 localities. Low genetic diversity characterized Argentine ant populations across South Africa. Nucleotide diversity π (standard deviation) was 0.001 (0.001) and haplotype diversity h (standard deviation) was 0.151 (0.048). The highest uncorrected sequence divergence separating specimens was 0.5 % (3 mutational steps) between ants collected from Elim2 and Stellenbosch. As is typical for mitochondrial DNA, the nucleotide composition of the COI sequences was AT-biased [A (32.05 %), C (17.96 %), G (13.09 %), T (36.89 %)] (Wolstenholme 1992, Crozier & Crozier 1993, Crease 1999, Althoff & Pellmyr 2002).

A close inspection of the COI sequence alignment indicated the presence of four variable sites resulting in five haplotypes (Table 5). Haplotype LH5 characterized 93 of the 101 specimens and was found at all localities included in the present study with the exception of Elim2, Porterville2 and Bloemfontein2 (see Table 3). Haplotype LH1, which characterized five

specimens, was restricted to the localities of Elim2, Porterville2 and Bloemfontein2. The haplotype network is shown in Fig. 5. All haplotypes are separated by single mutational differences and connect to the common haplotype LH5.

Table 5 Argentine ant haplotypes identified for the COI gene. Polymorphic nucleotide positions, distribution of haplotypes across South Africa and number of specimens characterized by haplotypes are indicated.

Haplotype	Base position				Locality	No.
	177	373	455	520		
LH1	A	G	T	G	Elim2, Porterville2, Bloemfontein2	5
LH2	G	A	T	G	Stellenbosch	1
LH3	A	G	C	G	Elim2	1
LH4	G	G	T	A	Stellenbosch	1
LH5	G	G	T	G	Majority of localities	93
Total						101

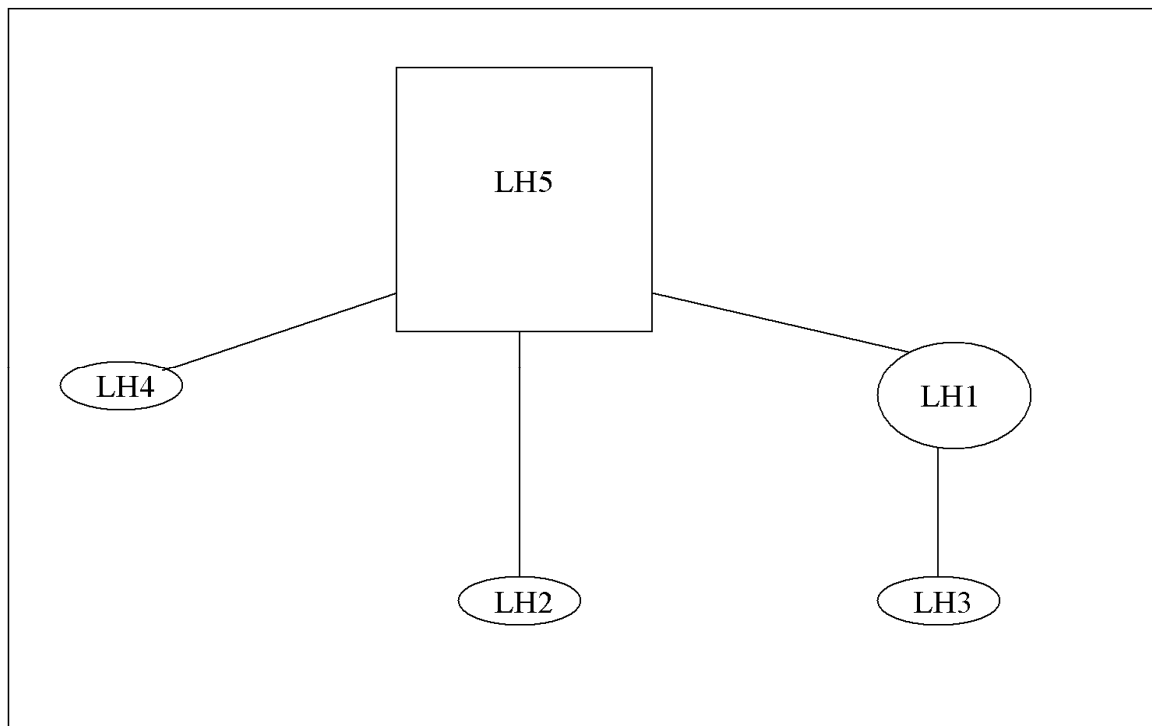


Fig. 5 Statistical parsimony network generated in TCS for Argentine ants sampled from across South Africa. This network is based on 618 bp of COI sequence for 101 ant specimens. The square (haplotype LH5) represents the presumed ancestral haplotype. The sizes of haplotypes are drawn according to their respective frequencies.

The rarefaction analysis (bootstrap estimator) suggested that many additional haplotypes might be found, but the Jack1 estimator suggested that only six are likely present in South Africa (Fig. 6). This is similar to the five haplotypes detected on the present study. Differences in sample size are not expected to influence the overall number of haplotypes observed since the analyses corrects for this variable. As such, the presence of rare alleles would not bias the expected number of six haplotypes.

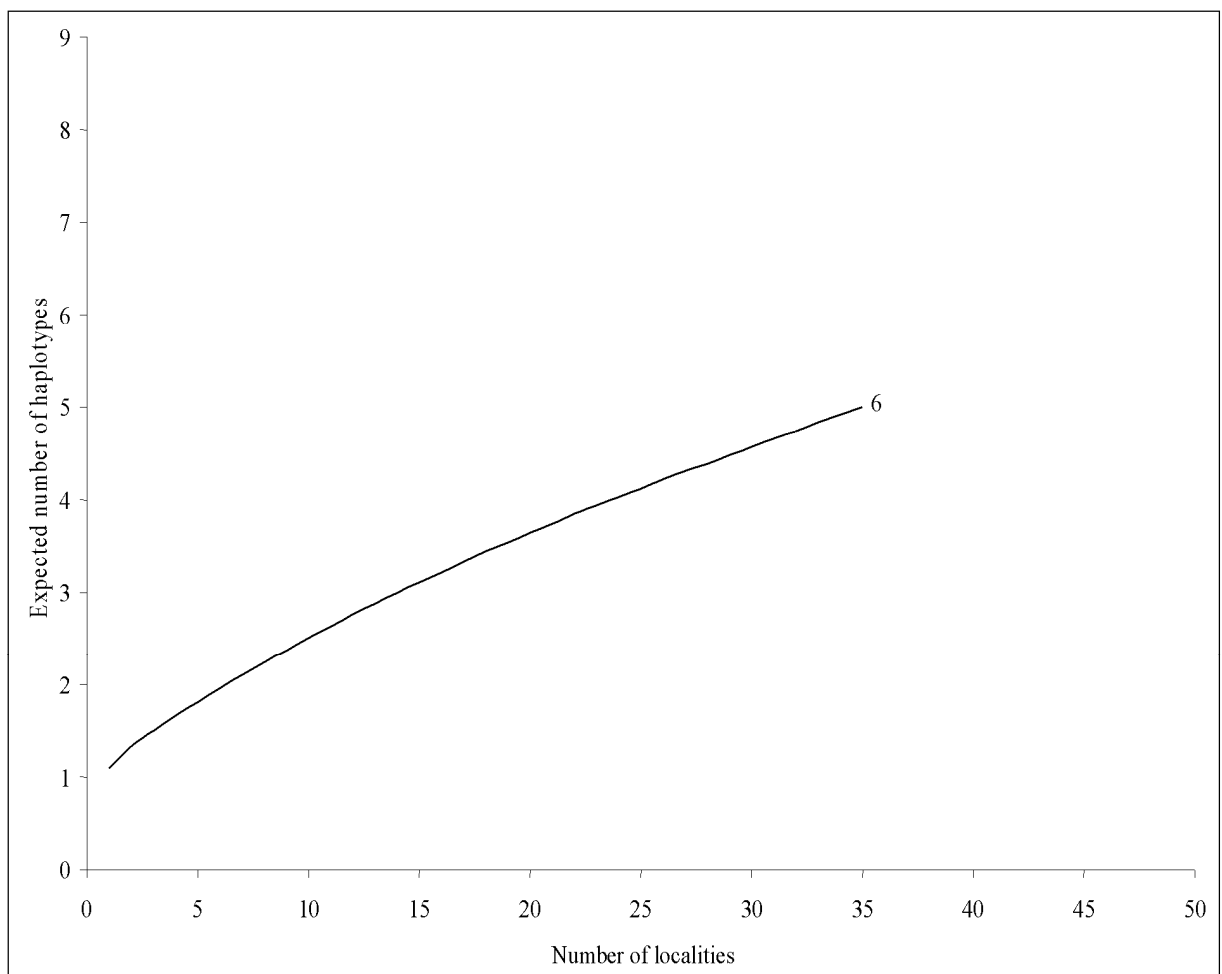


Fig. 6 Rarefaction curve showing the observed COI haplotypes and the number of sampling localities. The estimated number of Argentine ant haplotypes based on Jack1 is given in front of the curve.

3.4.1.2 Genetic differentiation

The analysis of molecular variance indicated significant structuring of genetic variation across South Africa with 54 % ($\Phi_{ST} = 0.54$, $P < 0.001$) of the variation accounted for by differences among sampling localities. The NMDS plot (Fig. 7) confirmed structuring with three distinct groups being evident. These were Elim2, Porterville2 and Bloemfontein2 (indicated in red), Stellenbosch (indicated in blue) and the remainder of localities (indicated in green). The stress value was < 0.1 indicating that the obtained configuration is reliable.

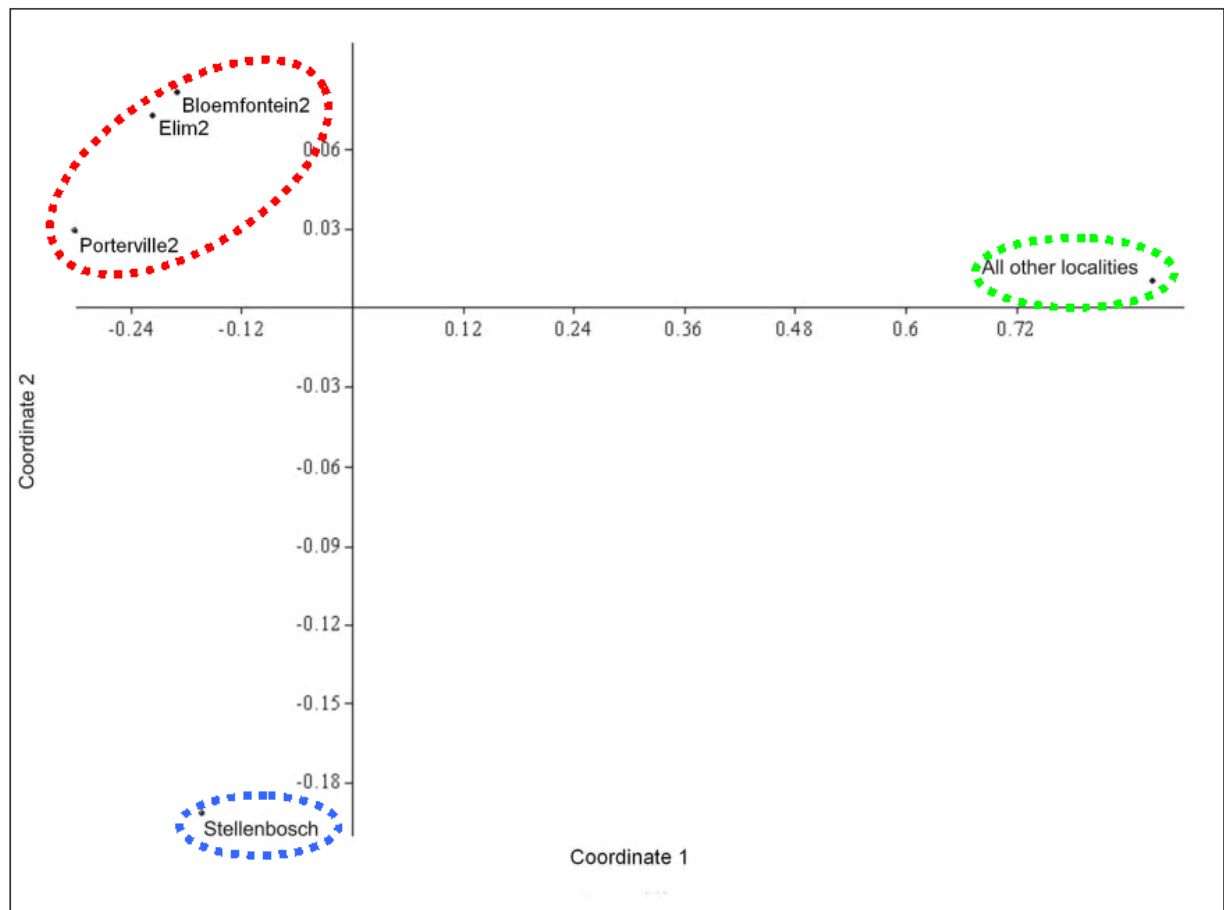


Fig. 7 Non-metric multidimensional scaling (NMDS) of Argentine ant genetic distances. The plot is based on 618 bp of COI gene sequences for 101 ant specimens from 35 geographic localities. Stress value S is < 0.1 .

3.4.1.3 Colony structure and composition

To verify the clusters detected with NMDS, these three groups were analyzed using an analysis of similarity (ANOSIM). The within clusters mean rank was 265.5 and the between clusters mean rank was 417.8 ($R = 0.512$, $P < 0.01$). It is possible for ANOSIM to be influenced if some of the groups are very different to the others. To verify that all three groups are indeed significantly differentiated, these three groups were compared in a pair-wise manner. The comparison between Stellenbosch and the rest of the localities were not significantly different (within cluster mean rank = 264, between cluster mean rank = 16, $R = -1$, $P = 1$). When testing whether the comparison between Stellenbosch and the group comprising Elim2, Porterville2 and Bloemfontein2, the R value was positive ($R = 1$), however, this was not a significant finding (within cluster mean rank = 2, between cluster mean rank = 5, $R = 1$, $P = 0.25$). The comparison between Elim2, Porterville2 and Bloemfontein2 and the rest of localities indicated significant differences between them (within cluster mean rank = 234.5, between cluster mean rank = 515, $R = 1$, $P < 0.001$). These results suggest that there are only two clusters that are significantly differentiated namely Elim2, Porterville2 and Bloemfontein2 compared with the rest of the localities (including Stellenbosch; within clusters mean rank was 250 and the between clusters mean rank was 547.5; $R = 1$, $P < 0.001$).

Gene flow was estimated at 0.05 individuals (queens) per generation between the two clusters (Elim2, Porterville2 and Bloemfontein2 as the first group and the remainder of the localities as the second group). This estimate is negligible small and suggests the absence of gene flow between the two groups.

3.4.1.4 Putative source population(s) for Argentine ants in South Africa based on cytochrome b data.

In an attempt to identify the (putative) source population(s) for introduction into South Africa, 738 bp of cytochrome b sequences were generated for 11 specimens from 11 localities across South Africa (see Table 3). Nucleotide diversity π (standard deviation) for this subset was 0.006 (0.035) and haplotype diversity h (standard deviation) was 0.890 (0.055). Four haplotypes (SA4 to SA7) were identified for the South African ants analyzed in the present study. These were separated by a maximum of 3 mutational steps.

The data generated in the present study were aligned to those provided by A. Suarez and N. Tsutsui (see Table 4). Since these sequences were of shorter length, the final dataset was truncated to 407 bp for 42 ants from 10 countries. This was done to avoid missing data in the final dataset given that missing data can have a profound effect on building networks and the retrieval of evolutionary relationship (see Joly *et al.* 2007). Following this truncation, haplotypes SA4 to SA7 (data from the present study) collapsed into a single haplotype. The three haplotypes (SA1, SA2 and SA3) identified by Tsutsui *et al.* (2001) collapsed into two haplotypes (SA1 from Cape Point was identical to SA3 from Caledon) with SA2 from Betty's Bay being most divergent. In total, 22 haplotypes were identified in Argentine ants from across the world.

The neighbour-joining tree, constructed from HKY + G (0.211) distances, is shown in Fig. 8. These results were largely congruent with results obtained from the haplotype network constructed in TCS (Fig. 9). The Brazilian haplotypes SJ1, SJ2, MV and PL2 could not be connected within the 95% confidence interval. The haplotype identified in the present study (SA4, SA5, SA6, and SA7) was identical to haplotypes from Argentina (Ocampo), Australia (Crozier) and New Zealand. This group (haplotype) was one step different from haplotype SA1

and SA3 and grouped with haplotypes from Brazil (PL1, Passo do Lontra) and Argentina (Ocampo and Rosario) (putative group circled in blue on Fig. 9). The South African haplotype SA2, identified by Tsutsui *et al.* (2001) did not cluster closely with any other haplotypes with 5 steps separating it from haplotype RIW (Italy) and SW2 (USA). Tsutsui *et al.* (2001) indicated that the South African samples also grouped with haplotypes from Buenos Aires; however, these samples were not available to us for inclusion.

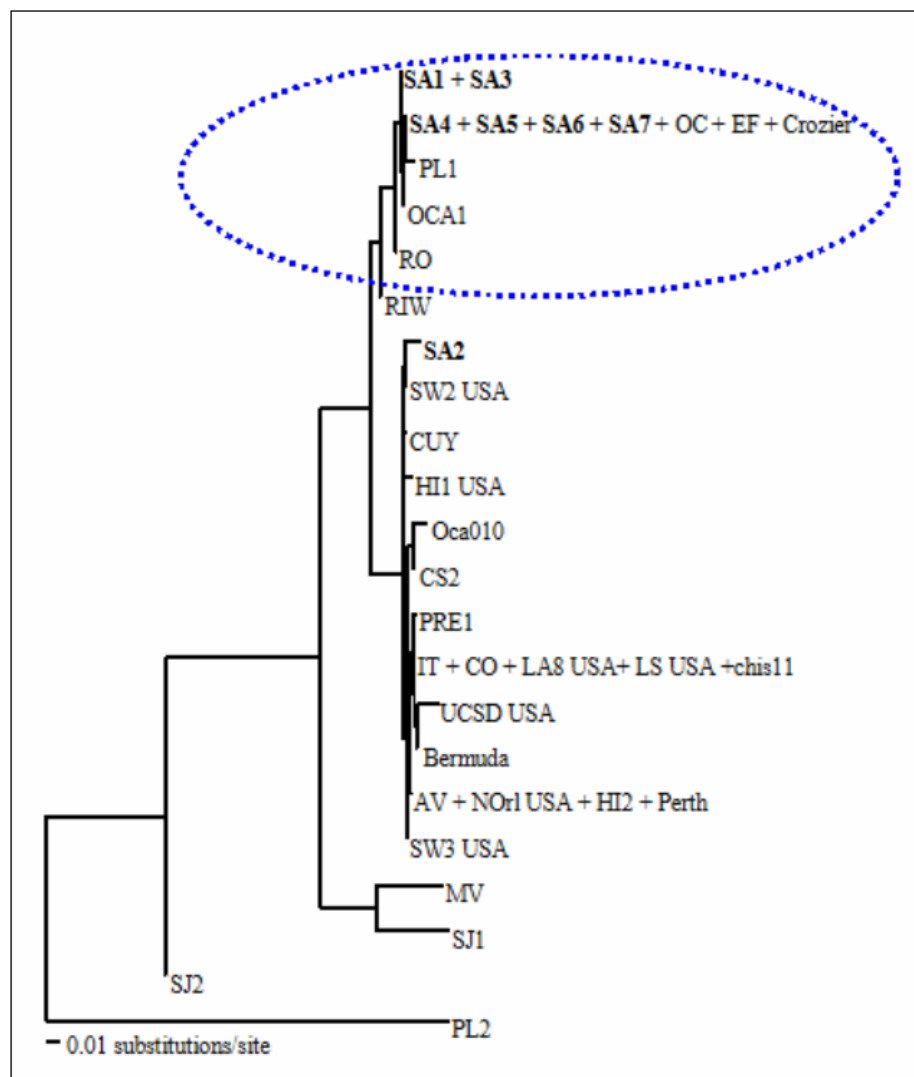


Fig. 8 Neighbour-joining tree based on 407 bp of cytochrome b. The tree was constructed from HKY+G corrected sequence divergences. South African haplotypes are indicated in bold. Two of the South African haplotypes grouped with those from Argentina, Brazil, New Zealand and Australia (indicated in blue). Refer to Table 4 for abbreviations.

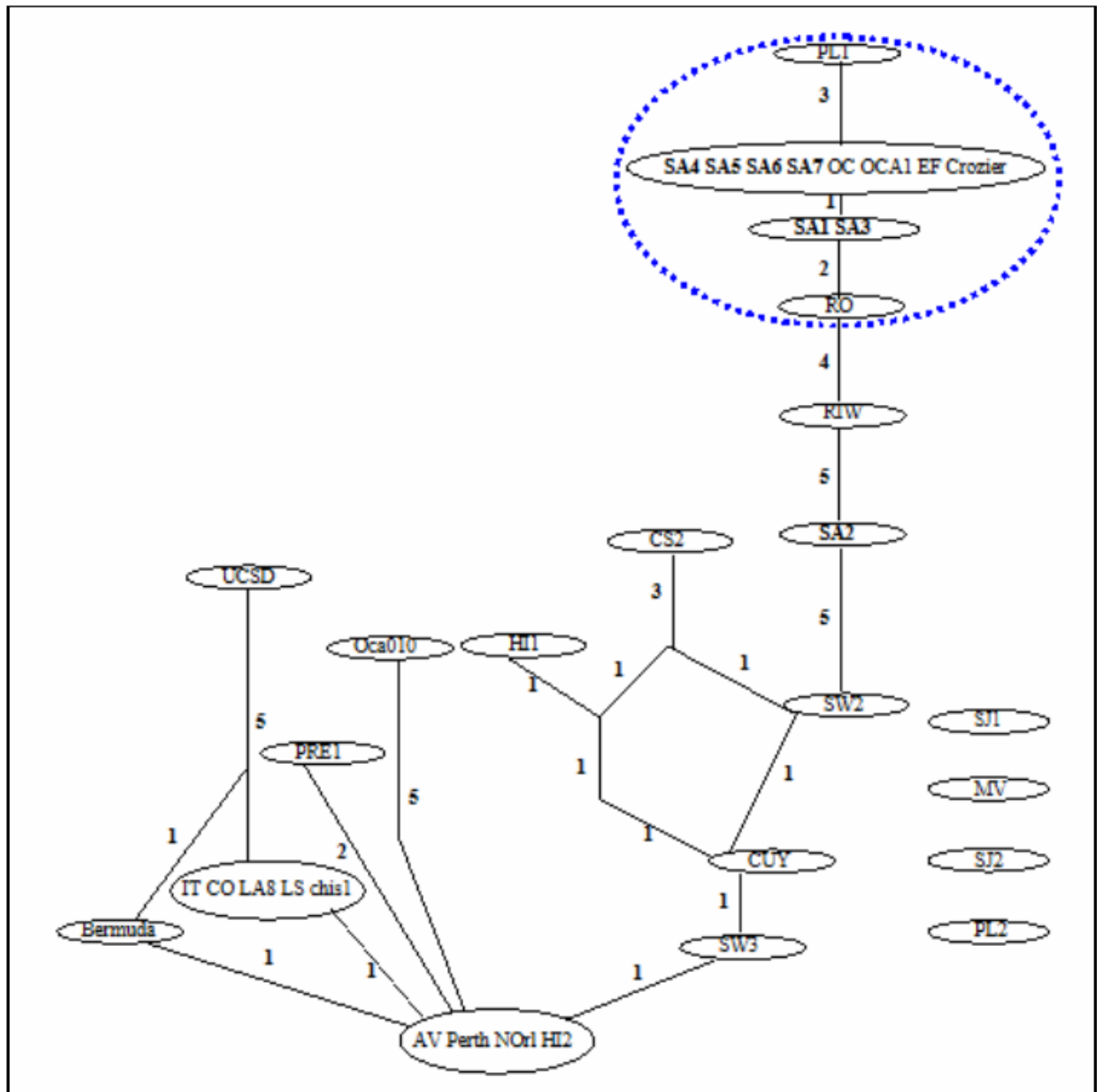


Fig. 9 The haplotype network constructed in TCS. Four haplotypes could not be connected within the 95% probability interval and these are shown on the side. Sizes of haplotypes are drawn according to their respective frequencies. Number of mutational steps between the haplotypes is indicated. South African haplotypes are shown in bold. Two of the South African haplotypes grouped with haplotypes from Brazil, Argentina, New Zealand and Australia and are shown in blue Refer to Table 4 for abbreviations.

3.4.2 Microsatellites

3.4.2.1 Genetic diversity

Standard measures of diversity (see Table 6) were calculated for 230 ants from 23 localities. There was no significant difference between observed (H_O) and expected (H_E) heterozygosities with Hardy Weinberg equilibrium across all loci and populations ($P = 1.0$). However, when performing locus specific tests, loci Lhum13, and Lhum35 did not conform to HWE ($P < 0.01$).

Table 6 Populations and samples (n) included in the microsatellite analyses. Mean number of alleles (A_O), observed (H_O), expected heterozygosities (H_E) and R_{ST} are reported.

Population	n	A_O	H_O	H_E	R_{ST}
Bloemfontein1	10	4.000 ± 1.528	0.517 ± 0.157	0.581 ± 0.157	0.149
Bellville	10	4.333 ± 2.055	0.600 ± 0.153	0.613 ± 0.187	0.121
Clanwilliam	10	3.500 ± 1.500	0.433 ± 0.137	0.552 ± 0.215	0.113
Elim2	10	2.667 ± 1.106	0.383 ± 0.267	0.361 ± 0.212	0.171
Demeul	10	4.167 ± 1.675	0.567 ± 0.125	0.593 ± 0.179	0.135
Laingsburg	10	3.333 ± 1.374	0.367 ± 0.213	0.473 ± 0.262	0.155
Nigel	10	3.833 ± 1.344	0.467 ± 0.243	0.520 ± 0.240	0.134
Nieuwoudtville	10	3.500 ± 1.384	0.383 ± 0.146	0.513 ± 0.227	0.149
Porterville2	10	3.000 ± 0.816	0.367 ± 0.205	0.456 ± 0.206	0.170
Richmond	10	3.833 ± 1.675	0.583 ± 0.168	0.555 ± 0.169	0.137
Stellenbosch	10	3.833 ± 2.034	0.500 ± 0.163	0.549 ± 0.200	0.139
Springbok	10	3.833 ± 1.344	0.400 ± 0.129	0.522 ± 0.211	0.145
Stormsvlei	10	4.667 ± 1.491	0.550 ± 0.150	0.607 ± 0.165	0.110
Tulbagh	10	4.500 ± 1.893	0.583 ± 0.196	0.604 ± 0.207	0.133
Uniondale	10	4.500 ± 2.363	0.450 ± 0.206	0.570 ± 0.200	0.122
Upington	10	3.500 ± 1.708	0.433 ± 0.243	0.592 ± 0.162	0.134
Aliwal North	10	3.500 ± 1.384	0.433 ± 0.309	0.570 ± 0.197	0.139
Lady Grey	10	3.667 ± 2.494	0.383 ± 0.313	0.418 ± 0.343	0.127
Bloemfontein2	10	2.167 ± 1.067	0.200 ± 0.191	0.234 ± 0.241	0.171
Lady Brand	10	3.500 ± 1.500	0.550 ± 0.335	0.503 ± 0.243	0.127
Betty's Bay	10	3.167 ± 1.167	0.367 ± 0.304	0.455 ± 0.291	0.109
Caledon	10	3.333 ± 2.055	0.383 ± 0.297	0.454 ± 0.332	0.138
Kogelberg. Res.	10	3.333 ± 1.972	0.367 ± 0.256	0.404 ± 0.280	0.145
Mean	10	3.600 ± 1.600	0.446 ± 0.096	0.510 ± 0.220	0.138

A rarefaction curve indicating the sufficiency of the number of samples collected to capture the level of microsatellite alleles variation in South Africa is shown in Fig. 10. Once more, the rarefaction curve asymptotically approaches the maximum of alleles available in

South Africa with Jack1 estimating the number of alleles at 67 (see the front of the curve) which is only marginally higher than the observed number of alleles (60, see Table 11 in the Appendix) reported in this study.

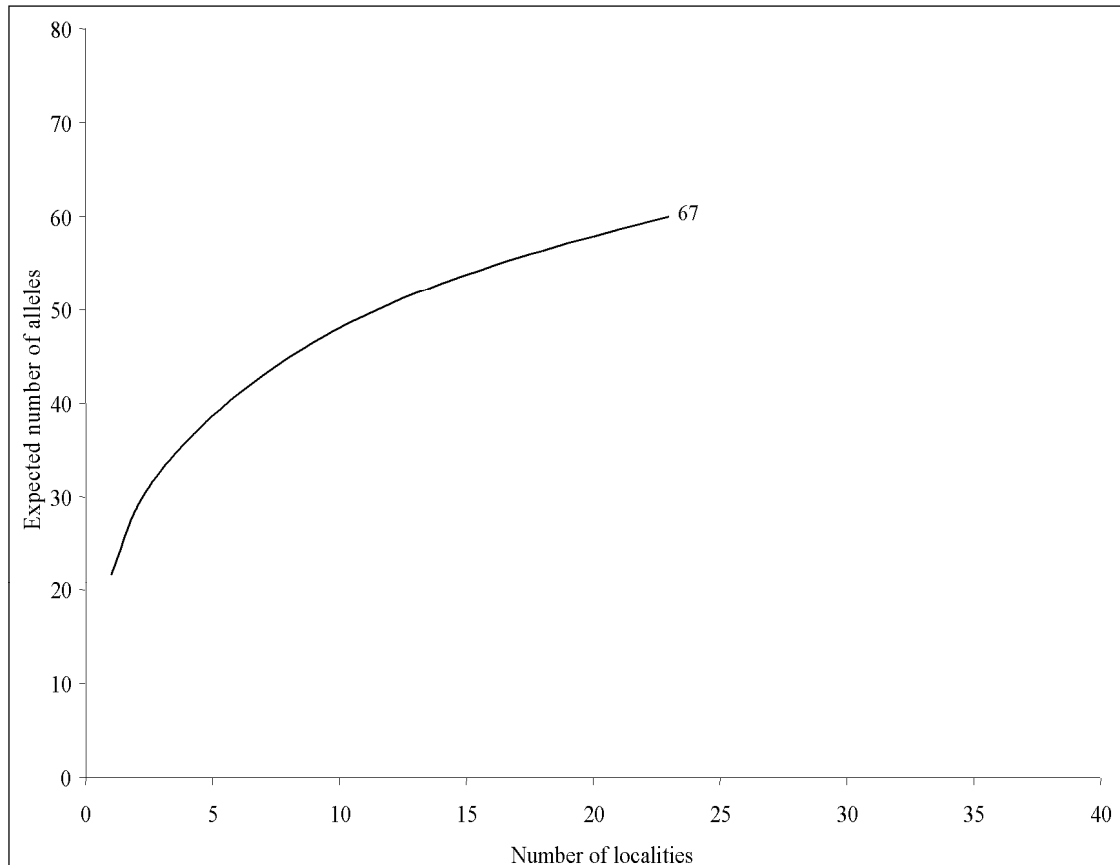


Fig. 10 Rarefaction curve of observed microsatellite allele variation and the number of localities sampled. Jack1-based estimated number of alleles is given in front of the curve.

Four loci (Lhum13, Lhum28, Lhum35, and Lhum52) contained null alleles with null allele frequencies ranging from -0.182 (for Lhum28 in Lady Brand) to 0.242 (for Lhum35 in Betty's Bay population; and for Lhum52 in the Upington population) (Table 7). Negative null allele's frequencies are the result of algorithm calculations but are nonetheless reported here. All null alleles detected at locus Lhum52 and one case involving locus Lhum35 (Betty's Bay) were due to scoring errors associated with stuttering as indicated by MICROCHECKER. The remaining six were neither associated with scoring errors nor with allele dropout. However, the

presence of null alleles in the Clanwilliam population may be due to significant inbreeding within the population ($R_{IS} = 0.45$, $P = 0.016$).

Table 7 Null allele frequencies for four loci and 12 populations of Argentine ants in South Africa. The negative frequencies are a result of the algorithm used (see discussion).

Pop	Lhum13	Null frequencies		
		Lhum28	Lhum35	Lhum52
Bloemfontein1	-0.086	0.152	0.031	-0.036
Clanwilliam	0.193	0.067*	0.078	0.091
Nieuwoudtville	0.099*	0.179	0.070	-0.036
Porterterville2	-0.013	-0.056	0.195*	0.155
Springbok	0.065	0.021	0.186*	-0.028
Uniondale	-0.053	0.169	0.155*	0.130
Upington	0.042	0.231	0.058	0.242*
Aliwal North	-0.166	0.185	0.195*	0.153
Lady Brand	-0.035	-0.188	0.086	0.153*
Betty's Bay.	-0.146	0.195*	0.242*	0.000
Caledon	-0.059	0.074	0.207*	0.000
Kogelberg Nature Reserve	-0.064	0.148*	0.050	0.000

* $P < 0.05$

Chi square results of observed allele frequencies and the adjusted allele frequencies (for the 12 populations) following the detection of null alleles were not significant meaning that population parameters estimation based on the original data are valid (see Table 8). Therefore the observed pattern is a reflection of the variation present in Argentine ant populations in South Africa.

Table 8 Comparisons between observed and adjusted allele frequencies for loci in populations where null alleles were detected. Significance was determined through a Chi square test with degrees of freedom (DF) and significance indicated (Ns = non significant).

Population	Locus	Df	X ²	Significance
Bloemfontein1	Lhum13	3	0.027	Non significant Ns
Clanwilliam	Lhum28	4	0.046	Ns
Nieuwoudtville	Lhum13	3	0.039	Ns
Porterville2	Lhum35	3	0.047	Ns
Springbok	Lhum35	5	0.043	Ns
Uniondale	Lhum13	3	0.034	Ns
	Lhum35	5	0.028	Ns
	Lhum52	2	0.020	Ns
Upington	Lhum13	3	0.069	Ns
	Lhum52	1	0.078	Ns
Aliwal North	Lhum13	2	0.042	Ns
	Lhum35	5	0.048	Ns
	Lhum52	1	0.027	Ns
Lady Brand	Lhum52	1	0.027	Ns
Betty's Bay	Lhum13	2	0.047	Ns
	Lhum35	4	0.077	Ns
Caledon	Lhum35	5	0.054	Ns
Kogelberg NR	Lhum13	3	0.026	Ns

3.4.2.2 Genetic differentiation

Microsatellite analysis supported the findings of the mitochondrial data analysis with significant structure detected across the landscape ($R_{ST} = 0.14$, $P < 0.001$). Much of the observed variation (86 %) was within populations. Isolation-by-distance was not detected across South Africa with mantel correlograms indicating no correlation between genetic distances (expressed as $R_{ST}/(1-R_{ST})$) and pairwise geographic distances ($r^2 = 0.005$, $P > 0.05$).

Similar to the mitochondrial DNA analysis, the NMDS plot based on microsatellite data grouped populations from Elim2, Porterville2 and Bloemfontein2 separate from the remainder of the localities (red circle) (Fig. 11). The stress value score S was < 0.150 .

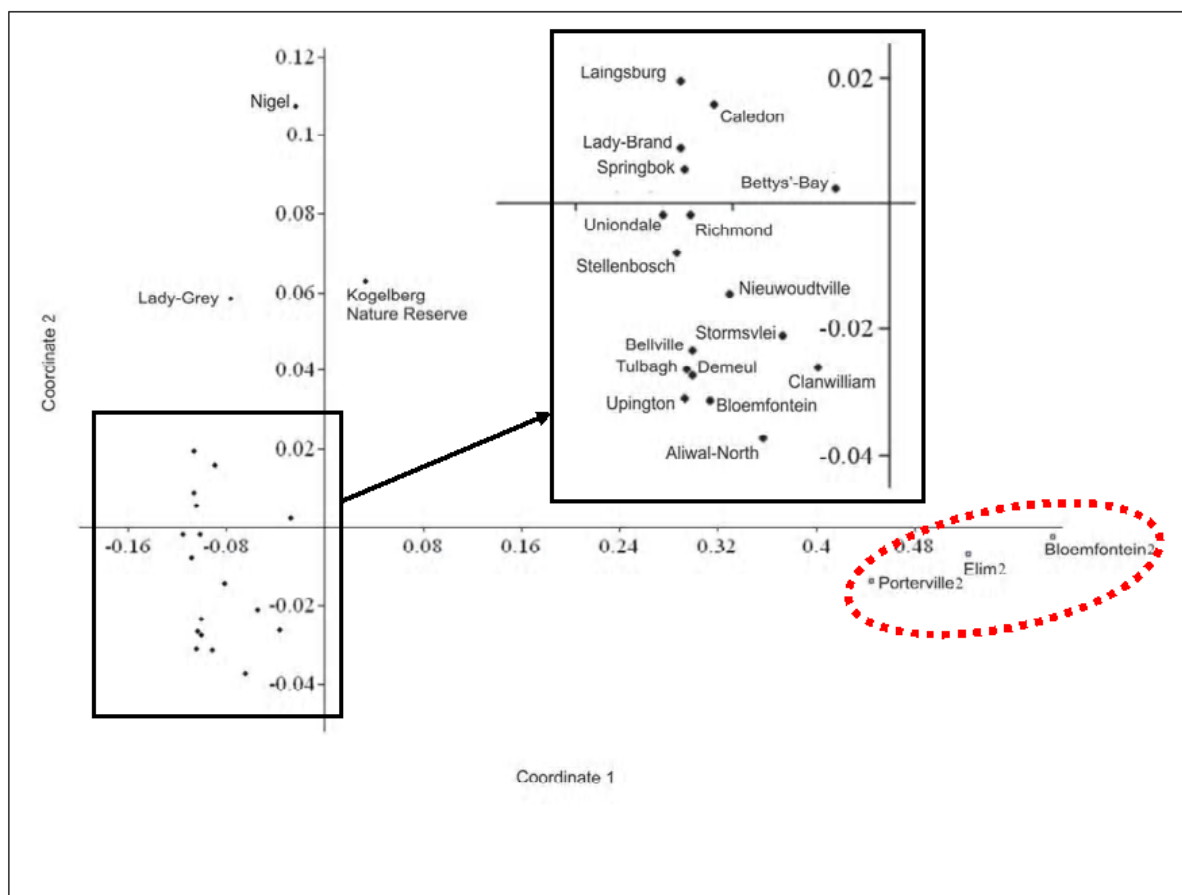


Fig. 11 Non-Metric Multidimensional Scaling of genetic distances of Argentine ants in South Africa showing the clustering of populations from Elim2, Porterville2 and Bloemfontein2 (Red). Stress value (S) was < 0.150 .

3.4.2.3 Colony structure and composition

Rare (frequency of alleles less than 0.1 in a population; Luikart *et al.* 1998) and unique alleles were present in nine (Bloemfontein1, Bellville, Elim2, Laingsburg, Nieuwoudtville, Springbok, Uniondale, Lady Grey and Caledon) of the 23 Argentine ant populations sampled in South Africa (Table 9). Six additional alleles were found to be present in all populations with the exception of Elim2, Porterville2 and Bloemfontein2. These three populations were further characterized by two unique alleles which were absent from the other populations.

Table 9 Rare alleles and their frequencies per Argentine ant population in South Africa. Unique alleles are listed per group.

Population	Locus	Allele size	Frequency
Rare alleles			
Bloemfontein1	Lhum11	117	0.004
	Lhum39	160	0.002
	Lhum39	188	0.002
Bellville	Lhum35	136	0.007
Elim2	Lhum11	119	0.002
	Lhum28	176	0.002
	Lhum39	146	0.002
Laingsburg	Lhum39	164	0.002
Nieuwoudtville	Lhum13	174	0.002
Springbok	Lhum28	186	0.002
	Lhum28	222	0.002
	Lhum28	234	0.002
Lady Grey	Lhum28	190	0.002
Caledon	Lhum13	174	0.002
Unique alleles			
Group comprising Bloemfontein2, Porterville2, Elim2			
	Lhum11	127	1.000
	Lhum28	182	1.000
Group comprising the remainder of the localities			
	Lhum11	111	1.000
	Lhum11	125	1.000
	Lhum13	178	1.000
	Lhum28	188	1.000
	Lhum35	110	1.000
	Lhum39	158	1.000

When performing ANOSIM based on microsatellite dataset with two groups specified (one group comprised populations from Elim2, Porterville2 and Bloemfontein2 and the other group represented populations from the remaining 20 localities), these two clusters were significantly different from each other. Within mean rank was (97), between mean rank was (223.5), and global $R = 1$ ($P < 0.001$). Once more this result suggests that the two clusters of populations might be from two different genetic pools.

When considering the two groups as defined for the ANOSIM analyses above, gene flow between these was estimated at 0.23 individuals (queens and drones) per generation. To test whether this pattern of gene flow is a true reflection of the presence of multiple colonies or rather typical of all Argentine ant populations across South Africa, overall gene flow was calculated for all populations within South Africa. In sharp contrast to the 0.23 individuals (queens and drones) migrating between the two groups, overall gene flow was estimated at 3.40 individuals (queen and drones) per generation.

Global relatedness for all 23 Argentine ant populations was not significantly different from zero ($r = 0.001 \pm 0.276$, $n = 23$, $t = 0.726$, $P = 0.475$). Cluster (colony)-wise relatedness were also not significantly different from zero r_1 [$(0.001 \pm 0.014$, $n = 20$, $t = 0.876$, $P = 0.392)$] and r_2 [$(-0.001 \pm 0.009$, $n = 3$, $t = -0.859$, $P = 0.482)$ respectively].

3.4.2.4 Demographic and evolutionary processes

3.4.2.4.1 Bottleneck test

Excesses in heterozygosity were detected in three Argentine ant populations from Clanwilliam, Springbok and Upington ($P < 0.05$). This would suggest these populations experienced a bottleneck sometime during their past (Table 10). However, the heterozygosity excess averaged over all populations was not significant.

Table 10 Heterozygosity excess and P-values of Argentine ant populations in South Africa. Bottlenecked populations are indicated by *.

Population	H _E excess	P-value
Bloemfontein1	0.596	0.505
Bellville	0.598	0.503
Clanwilliam	0.518	0.028*
Elim2	0.510	0.298
Demeul	0.608	0.188
Laingsburg	0.516	0.516
Nigel	0.508	0.061
Nieuwoudtville	0.535	0.500
Porterville2	0.491	0.250
Richmond	0.561	0.511
Stellenbosch	0.556	0.557
Springbok	0.587	0.041*
Stormsvlei	0.673	0.162
Tulbagh	0.626	0.469
Uniondale	0.612	0.463
Upington	0.523	0.033*
Aliwal North	0.535	0.514
Lady Grey	0.557	0.367
Bloemfontein2	0.422	0.389
Lady Brand	0.586	0.462
Betty's Bay	0.544	0.617
Caledon	0.640	0.448
Kogelberg Nature Reserve	0.547	0.390

3.4.2.4.2 Natural selection and population expansion

Moran's I values did not differ from zero accepting the null hypothesis of no autocorrelation. There was no genetic cline and no fine genetic structure in Argentine ant populations in South Africa (Fig. 12).

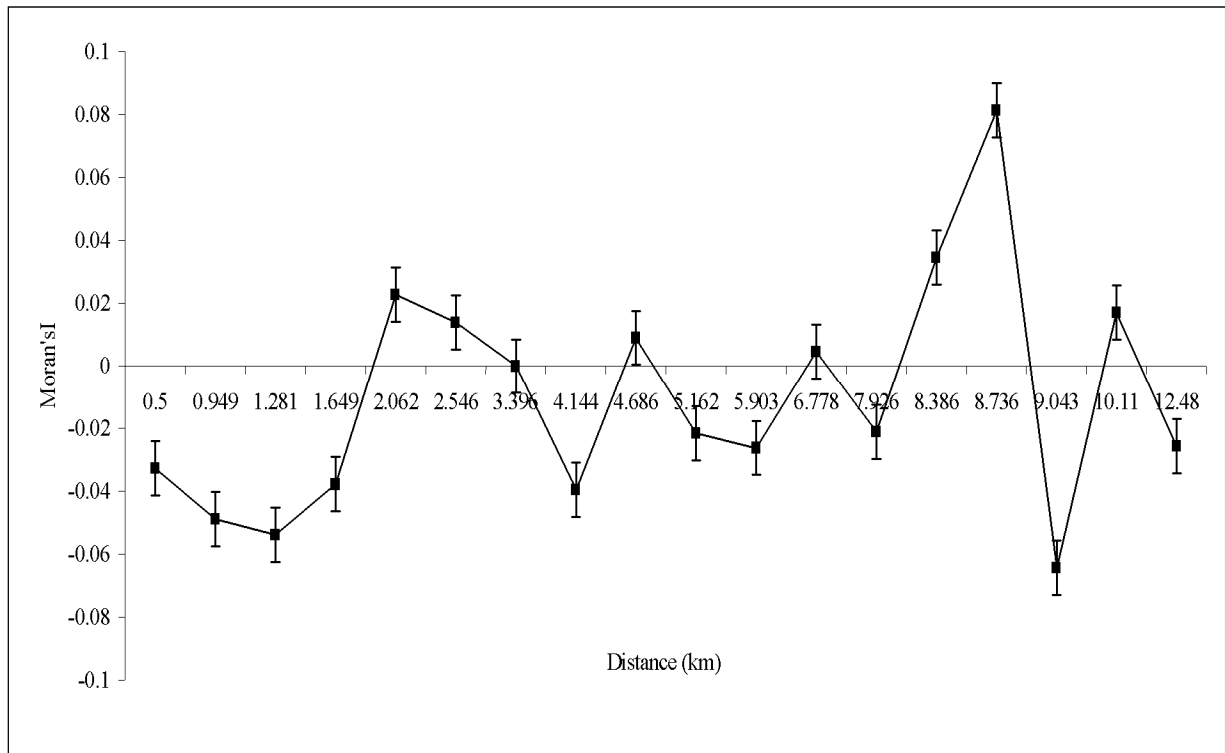


Fig. 12 Moran's I correlogram showing Moran's I coefficients plotted against geographic distance for Argentine ant populations in South Africa based on microsatellite data. Black squares represent autocorrelation coefficients of the Moran I statistics.

3.5 Discussion

3.5.1. Genetic diversity

Argentine ants in South Africa are characterized by relatively low levels of mitochondrial DNA variation. These low levels of variation can not be attributed to incomplete lineage sampling since rarefaction analyses indicated that COI haplotypes have been adequately sampled (see Fornia *et al.* 2007). Low levels of variation are not uncommon for Argentine ants across their introduced ranges (see for example Kaufmann *et al.* 1992) and have

been attributed to various factors including allelic cleansing or bottlenecks associated with introduction events. Slightly higher levels of variation were detected for cytochrome b which is consistent with this gene having a slightly faster mutation rate. Moderate levels of cytochrome b variation for Argentine ants in the native range were also reported by Pedersen *et al.* (2006) (but see Dreier *et al.* 2005 and Corin *et al.* 2007b which reported low levels of variation for Chile and New Zealand).

Moderate to high levels of overall microsatellite variation was found for ants in South Africa compared to the rest of the world (see for example Krieger & Keller 2000, Buczkowski *et al.* 2004). Expected heterozygosity in the present study was ($H_E = 0.51$) overall. This value is higher than that reported for several introduced colonies from across the world such as southern France ($H_E = 0.42$, Krieger & Keller 2000), California ($H_E = 0.44$, Buczkowski *et al.* 2004) and New Zealand ($H_E = 0.25$, Corin *et al.* 2007b). Although speculative, these high levels of variation may be as a result of high population numbers or multiple matings of queens (Gadagkar 1985, Ingram 2002a & b), and reflect the level of variation available in Argentine ants in South Africa as suggested by the rarefaction analysis for the microsatellites data (Leberg 2002). It is important to note that Stellenbosch represent the first locality in South Africa where Argentine ants were documented from, and high heterozygosity, at least in this locality, may be a remnant signature of the site of introduction into South Africa; this is further substantiated by a unique mitochondrial COI haplotype.

Populations from Elim2 ($H_E = 0.36$), Bloemfontein2 ($H_E = 0.23$) and Porterville2 ($H_E = 0.46$) were characterized by lower levels of variation. Although inbreeding and bottlenecks could account for the lower levels of genetic variation, this was conclusively ruled out in this study since the coefficients of inbreeding as well as bottleneck tests for these three populations

were not significant ($p > 0.05$). Low levels might, at least in part, be accounted for by lower effective population sizes.

3.5.2 Genetic differentiation

The findings of this study clearly demonstrate that Argentine ants in South Africa are genetically structured. This structuring is not in terms of isolation-by-distance but due to presence of two distinct populations that may have arisen from two different introductions. Both mitochondrial and nuclear DNA support the presence of two groups/clusters corresponding to the localities of Elim², Porterville² and Bloemfontein² as a cluster distinct from the remainder of localities included in the study. These might represent two colonies with behavioural data indicating high levels of aggression between the Elim population compared to other populations in the Western Cape (Theresa Wossler and Natasha Mothapo personal communication). To confirm that these genetic clusters do indeed represent distinct colonies, additional behavioural assays and cuticular hydrocarbon profiles (Tsutsui *et al.* 2001, Buczkowski *et al.* 2004, Jaquiéry *et al.* 2005, Corin *et al.* 2007a) should be collected.

An important finding to emerge from this study is that the genetic clusters (possibly colonies) in South Africa are not continuously distributed but rather show a disjunct and interspersed distribution pattern. For example, two geographic sites, separated by ~ 4 kilometers, were included for the localities of Elim, Porterville and Bloemfontein. In each of these cases were the two sampling sites characterized by vastly different genetic patterns. This is not a finding unique to South Africa and agrees with Giraud *et al.* (2002) and Tsutsui *et al.* (2003) who reported that some colonies are small, isolated and found between larger colonies in Southern Europe and California respectively. Pedersen *et al.* (2006) similarly found that some colonies in the native range of Argentina are not continuously distributed but are

interspersed by other colonies (see also Tsutsui *et al.* 2000, Tsutsui & Case 2001). It has been argued by Wetterer and Wetterer (2006) that the presence of single large colonies (so-called super colonies) may prevent the introduction and establishment of secondary colonies. However, from our own sampling efforts as well as the work done by Luruli (2007) it is clear that the distribution of Argentine ants in South Africa is by no means continuous. Although these ants have largely reached the extend of their distribution, there remains large areas within this range that is unoccupied (Melodie McGeoch, personal communication). This might explain why two genetically distinct colonies can occur in geographical proximity. Why some colonies remain relatively small and isolated while others become large, quasi-continuously distributed and occupy expansive ranges raises an important question namely why are some Argentine ant colonies small and restricted in their distribution and others are not? Is it because they are ecologically and evolutionary constrained? Answers to these questions can influence where to direct or concentrate control efforts.

The grouping of populations from different localities and provinces into clusters of closely related populations suggests considerable transfer of Argentine ants between localities within and across South Africa. Two factors might have contributed to this. First, the introduction of the Argentine ant into Cape Town coincided with the onset of the Anglo-Boer War at the turn of the century (Skaife 1953). At that time, Cape Town was receiving British troop reinforcements and supplies such as fodder for the horses as well as construction material for building barracks and supply routes for the troops to facilitate goods movement between Cape Town and the frontlines (de Villiers 1984, Vernon 1985) (Fig. 13). Second, contemporary factors such as agricultural activities and an expanding nursery trade may largely facilitate the current movement of ants across South Africa.

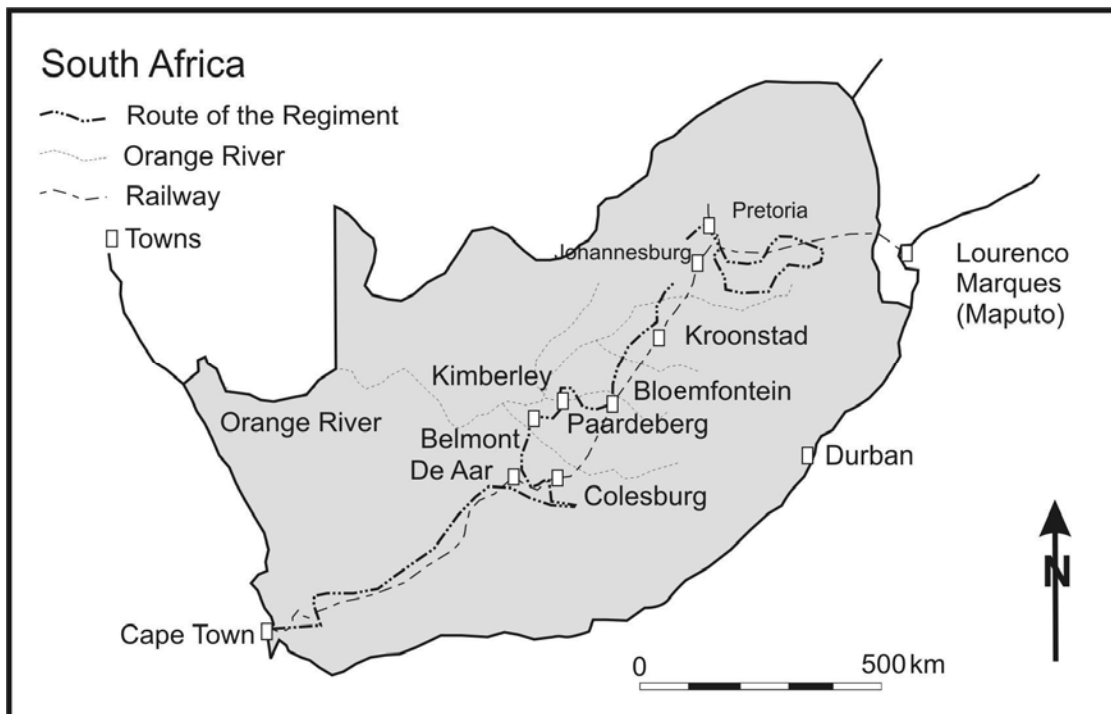


Fig. 13 Map of British troop movements during the Anglo-Boer War redrawn from Vernon (1985). Path followed by the British troops is indicated by the truncated black line. Railway lines and important towns at the time are indicated.

No isolation-by-distance was observed in South Africa at both local and regional scales. Although this might be the result of the present study being based on workers rather than queens (Reuter *et al.* 2001 reported isolation-by-distance when analyses are based on queens alone since they are philanthropic), no isolation-by-distance have similarly been reported for introduced populations of this species in the USA (Buczkowski *et al.* 2004 but see Ingram & Gordon 2003) and Europe (Jaquiéry *et al.* 2005). Furthermore, our finding is in line with the suggestion that natural barriers to gene flow may be playing a limited role, as the dispersal of Argentine ants over long distances is almost certainly assisted by humans (Suarez *et al.* 2001, Roura-Pascual *et al.* 2004). This is based on the notion that if dispersal in the South African Argentine ants were predominantly by budding (queens) and flight (males) as is the case in the native range (Tsutsui & Case 2001), there would have been a decrease in gene flow with increasing distance.

3.5.3 Colony structure and composition

The assertion by Tsutsui *et al.* (2001) that Argentine ants in South Africa might be a result of multiple introductions was largely confirmed in this study. The clear grouping of Argentine ant mitochondrial haplotypes into two major clusters was corroborated by the microsatellite data. Divergence in both allelic frequencies and composition is in line with Suarez & Tsutsui (2008) argument that populations introduced several times from sources in their native range would be characterized, among, others by differences in allelic frequencies and unique alleles.

Argentine ants from South Africa conform to the trend found for ants from across the world in that an overall low (non-significant) mean relatedness among populations and within clusters (colonies) was found (Kaufmann *et al.* 1992, Krieger & Keller 2000, Ingram & Gordon 2003). The observed low levels of relatedness across populations and clusters may be explained by multiple queens with multiple matings in different colonies (Gadagkar 1985, Reuter *et al.* 2001, Vega & Rust 2001, Ingram & Gordon 2002a & b). The consequences of low overall inter-population and intra-colony relatedness are two fold. First, Tsutsui *et al.* (2003) have argued that populations that differ in levels of genetic diversity tend to be aggressive towards each other. The rationale is that populations that are highly related will tend to suffer from inbreeding which will reduce genetic diversity. The findings from this study report overall low levels of relatedness suggesting that relatedness may not be playing a significant role in the levels of genetic structure among the Argentine ant populations. Second, the observed inbreeding in some Argentine ant populations in South Africa may not only be due to the group living of related individuals (Keller & Fournier 2002). This is because inbred and non-inbred populations have been found to have similar levels of relatedness that did not differ from zero (Ross 2001, Pedersen *et al.* 2006). Furthermore, the coefficients of relatedness

reported in this study may be high if compared to native populations suggesting relatedness may not necessarily constitute a problem for the kin selection theory (see Tsutsui & Suarez 2003, Suarez *et al.* 2008).

3.5.4 Putative source population(s) for Argentine ants in South Africa

Cluster analyses indicated that Argentine ants in South Africa group with ants from Argentina (Ocampo and Rosario) as well as from Brazil (Passo do Lontra). Tsutsui *et al.* (2001) further indicated Buenos Aires (Argentina) as potential source for ants in South Africa (data from Buenos Aires were not available for the present study).

Argentine ants in South Africa also shared a haplotype with ants from Australia and New Zealand (Tsutsui *et al.* 2001, Ward *et al.* 2006, Corin *et al.* 2007b). This raises the important point that introductions of ants across the world may not necessarily be from source populations in their native range but may be introduced from other introduced populations. Indeed, Corin *et al.* (2007a) implicated mainly Australia and to a lesser degree South Africa as sources of Argentine ants in New Zealand.

3.5.5. Demographic and evolutionary processes

Evidence for bottlenecks was detected in three Argentine ant populations in South Africa. Although a founder event (the introduction of relatively few individuals) may account for the bottlenecks, the reduction in size of an initially healthy population may also result from ecological factors such as severe weather conditions (Roura-Pascual *et al.* 2004, Menke & Holway 2006). This finding agrees with several other investigators who reported bottlenecks in Argentine ants in other introduced areas (Tsutsui *et al.* 2001, Giraud *et al.* 2002, Buczkowski *et al.* 2004).

The lack of isolation-by-distance may point to long-jump dispersal (such as would result through human-mediated dispersal) as playing a role in the observed genetic pattern (Meixner *et al.* 2002, Therriault *et al.* 2005, Cameron *et al.* 2008). Range expansion or natural dispersal in Argentine ants may follow a stepping stone model or radial model (Ingram & Gordon 2003, Liebhold & Tobin 2008). Under the stepping stone model, a naturally expanding population will be characterized by decrease in the number of rare alleles and the overall level of genetic diversity with increasing distance. Radial mode of dispersal (Liebhold & Tobin 2006) will result in a star-like haplotype network with the presumed old or ancestral haplotype situated in the middle (see Slatkin & Hudson 1991, Myburgh *et al.* 2007). The genetic pattern observed for Argentine ants across South Africa does not match any of these two models and natural range expansion may therefore not have played a major role in the observed pattern since no parallel patterns of spatial genetic structure could be detected (Sokal & Oden 1978a & b, Kennington *et al.* 2003, Roff 2003, Stone *et al.* 2003).

No evidence was found to suggest that natural selection is acting on Argentine ant populations in South Africa. Assuming that the methodology used in the present study is sensitive enough to detect selection, the lack of genetic clines as well as fine scale genetic structure in the studied populations indicates no spatially varying selection (Slatkin & Arter 1991, Kennington *et al.* 2003, Stone *et al.* 2003).

The fact that spatial autocorrelation analysis could not reveal the action of selection does not mean that the search for the role of selection in shaping the observed patterns of genetic structure should be halted. Three reasons would indicate that natural selection may indeed play a role in shaping the variation across South Africa. First, rare alleles were observed for all loci but one (Table 9). Second, several localities were fixed for specific alleles

suggesting that these populations might have experienced negative selection. Third, our analyses did not include morphological measurements. A morphological analysis of the studied population may reveal cline in body size of the Argentine ant in South Africa which may be due to selection (see Huey *et al.* 2000, De Jong & Bochdanovits 2003).

3.6 Lessons from this study for the control of Argentine ants in South Africa

The results from this study have several implications for the way Argentine ant populations in South Africa are managed. Some mitochondrial haplotypes and nuclear genotypes are widespread throughout South Africa indicating considerable movement of ants across large geographic areas. These movements might be largely human-mediated through agriculture and the nursery trade. In addition, mitochondrial and nuclear results indicate the presence of distinct genetic groups which might correspond to two colonies. In addition, several putative source populations from geographically diverse localities in the native range are implicated. These findings would hold implications should biocontrol measures be considered in the future. It is further important to consider that South Africa may act as a potential source of introduction for other countries. For example, a recent study implicated South Africa (and Australia) as source for the introduction of Argentine ants into New Zealand. Care should therefore not only be taken regarding the import of potential invasive species but also not to export these.

Chapter 4: Summary and avenues for future research

Information on the population genetics of introduced solitary and social insects across South Africa is needed. This study attempts to provide such information on two species with two different modes of introduction (intentional and unintentional) and two different life histories (solitary and social) into South Africa. The findings from this study may serve as baseline information needed to promote management and control efforts for these introduced species. In addition, this study provides possible scenarios and mechanisms underlying the observed patterns.

4.1 *Trichilogaster acaciaelongifoliae* (Chapter 2)

4.1.1 Summary of the main findings

The bud gall-forming wasp *T. acaciaelongifoliae* was introduced into South Africa from Australia as a biocontrol agent to curb the spread of the invasive long-leaved wattle *A. longifolia*. Two separate introduction events took place early in the 1980s: the first mainly comprised localities in and around Stellenbosch (Western Cape Province) with the second event involving the release of subsequent generation derived from some 14,000 wasps introduced to several localities in the Western and Eastern Cape Provinces. The wasp has since spread throughout South Africa and has also shifted from the target host to the Sally wattle (*A. floribunda*) that is commonly used as an ornamental plant.

Mitochondrial COI sequences were generated for wasps collected from across the distribution in South Africa. Sequence data revealed low genetic diversity within South Africa with only three haplotypes detected for 53 wasps from 23 localities. Two haplotypes were wide-spread and found at most sampling localities with the third haplotype characterizing a single individual from Grahamstown (one of the sites of original introduction). Populations of

T. acaciaelongifoliae were neither geographically nor host specifically differentiated. The observed pattern in *T. acaciaelongifoliae* may be largely due to human-mediated jump-dispersal. However, selection for specific (optimal) haplotypes may also account for the success of the two common haplotypes; unfortunately we could not unequivocally test this hypothesis in the present study.

4.1.2 Implications of the study for *T. acaciaelongifoliae* and future directions

Records of human-mediated dispersal of biological control agents are important because human-facilitated dispersal can affect levels of genetic variation and its distribution across the range. Therefore, if members of the public are involved in the release of the biological control agents, then members of the public and biological control authorities should keep records of the released populations and the released sites. This would enable the close monitoring and management of the released agents, both at small and large spatial scales.

Future work on the bud gall-forming wasp in both native Australian and introduced South African range should include the use of wasp-specific hypervariable genetic markers such as microsatellites. The use of more sensitive markers may possibly reveal genetic patterns that remained obscured in the present study.

4.2 *Linepithema humile* (Chapter 3)

4.2.1 Summary of the main findings

The Argentine ant *L. humile* was accidentally introduced into South Africa in animal fodder imported from South America. The initial introduction as well as early spread is believed to be linked to the Anglo-Boer war at the turn of the previous century. The spread of

Argentine ants across South Africa is largely through human-mediated jump-dispersal through agricultural and nursery trade.

Across its native range, the Argentine ant forms small colonies (dispersed over tens to hundreds of meters) with inter-nest aggression. In sharp contrast, across its introduced range this species form large super colonies that may cover several thousands of kilometres with no inter-nest aggression; a factor that greatly increases its potential and success as a world invader. An earlier study by Tsutsui *et al.* (2001) found divergent haplotypes in South Africa leading them to suggest that Argentine ants in South Africa may be a result of multiple introductions with the establishment of more than one colony. To investigate this and to determine putative source populations for Argentine ants in South Africa, mitochondrial COI and cytochrome b sequences as well as microsatellite data were generated for 101 (from 35 localities), 11 (from 11 localities) and 230 ants (from 23 localities) respectively.

The results from the mitochondrial COI and cytochrome b sequences were largely congruent with those obtained from the microsatellite markers. Although mitochondrial data displayed lower levels of genetic diversity compared to the microsatellite markers, both marker systems suggested significant partitioning of genetic variation across the South African distribution range. Cytochrome b sequences indicated that ants in South Africa originated from at least three source populations (two in Argentina and a third in Brazil) with Tsutsui *et al.* (2001) suggesting an additional site in Argentina.

4.2.2 Implications of this study for the control of Argentine ants in South Africa

Argentine ant males are vital for the maintenance of genetic variation within populations and colonies. This is because gene flow in Argentine ants is male biased. Although

male Argentine ants are short-lived, control efforts should aim to prevent successful matings from taking place. This will reduce population growth and consequently lead to the gradual control of this pest. Within South Africa, it would appear that human-aided dispersal of Argentine ants as well as the number of introductions was considerable, both within and between the provinces. This observation calls for more strict local control measures. Strict enforcement of control measures will reduce the number of fresh introductions.

4.2.3 Future directions

Future work should include analysis of male genetic structure and relatedness alongside that of queens and workers and test the hypothesis that gene flow in Argentine ants is male-biased. Furthermore, comparative study of genetic variation and morphological variation between the small and expansive colonies across the introduced ranges may reveal whether Argentine ant populations differ in their degree of invasiveness.

References

- Allendorf FW, Lundquist LL, (2003) Introduction: Population biology, evolution and control of invasive species. *Conservation Biology*, **17**, 24-30.
- Alstad D (1998) Population structure and the conundrum of local adaptation. In: Population Structure and Conundrum of Local Adaptation (eds. Mopper S, Strauss SY), pp. 3-21. Chapman & Hall, New York.
- Althoff DM, Pellmyr O (2002) Examining genetic structure in a bogus yucca moth: a sequential approach to phylogeography. *Evolution*, **56**, 1632-1643.
- Ayala FJ (1976) Molecular evolution. Sinauer Association, Inc., Sunderland, Massachusetts.
- Baer CF, Tripp DW, Bjorksten TA, Antolin MF (2004) Phylogeography of a parasitoid wasp (*Diaeretiella rapae*): no evidence of host-associated lineages. *Molecular Ecology*, **13**, 1859-1869.
- Baker DA, Loxdale HD, Edwards OR (2003) Genetic variation and founder effects in the parasitoid wasp, *Diaeretiella rapae* (M'Intosh) (Hymenoptera: Braconidae: Aphidiidae), affecting its potential as a biological control agent. *Molecular Ecology*, **12**, 3303-3311.
- Ball SL, Armstrong KF (2006) DNA barcodes for insect pest identification: a test case with tussock moths (Lepidoptera: Lymantriidae). *Canadian Journal of Forest Resources*, **36**, 337-350.
- Ballard JWO, Kreitman M. (1995). Is mitochondrial DNA a strictly neutral marker? *Trends in Ecology and Evolution*, **10**, 485-488.
- Ballard JWO, Rand DM (2005) The population biology of mitochondrial DNA and its phylogenetic implications. *Annual Review of Ecology, Evolution and Systematics*, **36**, 621-642.
- Bazin E, Glomming S, Galtier N (2006) Population size doesn't influence genetic diversity in animals. *Science*, **312**, 570-572.
- Behura SK (2006) Molecular marker systems in insects: current trends and future avenues. *Molecular Ecology*, **15**, 3087-3113.

- Belkhir K, Dawson KJ, Bonhomme F (2006) A comparison of rarefaction and Bayesian methods for predicting the allelic richness of future samples on the basis of currently available samples. *Journal of Heredity*, **97**, 483-492.
- Blier PU, Dufresne F, Burton RS (2001) Natural selection and evolution of mitochondrial DNA-encoded peptides: evidence for intergenomic co-adaptation. *Trends in Ecology and Evolution*, **17**, 400-406.
- Boato A, Battisti A, (1996) High genetic variability despite haplodiploidy in primitive Sawflies of the genus *Cephaleida* (Hymenoptera, Pamphiliidae). *Cellular & Molecular Life Sciences*, **52**, 516-521.
- Brookfield JFY (1996) A simple new method for estimating null allele frequency from heterozygote deficiency. *Molecular Ecology*, **5**, 453-455.
- Brose U, Martinez ND, Williams RJ (2003) Estimating species richness: sensitivity to sample coverage and insensitivity to spatial patterns. *Ecology*, **84**, 2364-2377.
- Brower AVZ (1994) Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the Natational Academy of Sciences of the USA*, **91**, 6491-6495.
- Boucher C, Stirton CH (1978) Long leaved wattle *Acacia longifolia* (Andr.) Willd. In Plant Invaders, Beneficial but Dangerous, pp. 49-98. Ed. Stirton CH. Department of Nature and Environmental Conservation of the Cape Provincial Administration. Cape Town: ABC Press.
- Buczkowski G, Vargo E L, Silverman J (2004) The diminutive supercolony: The Argentine ants of the Southeastern United States. *Molecular Ecology*, **13**, 2235-2242.
- Cameron EK, Bayne EM, Coltman DW (2008) Genetic structure of invasive earthworm *Dendrobaena octaedra* in the boreal forest of Alberta: insights into introduction mechanisms. *Molecular Ecology*, **17**, 1189-1197.
- Carrol SP, Hendry AP, Reznick DN, Fox CW (2007) Evolution on ecological time-scales. *Functional Ecology*, **21**, 387-397.
- Chiotis M, Jermin LS, Crozier RH (2000) A molecular framework for the phylogeny of the ant subfamily Dolichoderinae. *Molecular Phylogenetics and Evolution*, **17**, 108-116.

- Christian CE (2001) Consequences of a biological invasion reveal the importance of mutualism for plant communities. *Nature*, **413**, 635-638.
- Clarke KR, Green RH (1988) Statistical design and analysis for a 'biological effect' study. *Marine Ecology Progress Series*, **46**, 213-226.
- Clement M, Posada D, Crandall K (2000) TCS: A computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657-1660.
- Colwell RK (2005) EstimateS: Statistical estimation of species richness and shared species from samples. Version 7.5. User's Guide and Application published at: <http://purl.oclc.org/estimates>.
- Colwell RK, Coddington JA (1994) Estimating terrestrial biodiversity through extrapolation. *Philosophical Transactions of the Royal Society of London, Series B*, **345**, 101-118.
- Confal nieri VA, Sequeira AS, Todaro L, Vilardi JC (1998) Mitochondrial DNA and phylogeography of the grasshopper *Trimerotropis pallidipennis* in relation to clinal distribution of chromosome polymorphism. *Heredity*, **81**, 444-452.
- Corin SE, Abbot KL, Ritchie PA, Lester PJ (2007b) Large scale unicoloniality: The population and colony structure of the invasive Argentine ant (*Linepithema humile*) in New Zealand. *Insects Society*, **54**, 275-282.
- Corin SE, Lester PJ, Abbot KL, Ritchie PA (2007a) Inferring historical introduction pathways with mitochondrial DNA: The case of introduced Argentine ants (*Linepithema humile*) into New Zealand. *Diversity and Distributions*, **13**, 510-518.
- Cornuet JM, Luikart G. (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, **144**, 2001-2014.
- Corry JS, Myers JH (2000) Direct and indirect effects of biological control. *Trends in Ecology and Evolution*, **15**, 137-139.
- Costermans LF (1981) Native Trees and Shrubs of South Eastern Australia. Rigby, Adelaide, N. S. W.
- Crease TJ (1999) The complete sequence of the mitochondrial genome of *Daphnia pulex* (Cladocera: Crustacea). *Gene*, **233**, 89-99.
- Crispo E, Hendry AP (2005) Does time since colonization influence isolation by distance? A meta-analysis. *Conservation Genetics*, **66**, 665-682.

- Crozier RH (1977) Evolutionary genetics of the hymenoptera. *Annual Review of Entomology*, **22**, 263-288
- Crozier RH, Crozier YC (1993) The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization. *Genetics*, **133**, 97-117.
- Dawnay N, McEwing R, Thorpe RS, Ogden R (2008) Preliminary data suggests distinctiveness of gyr and saker falcons. *Conservation Genetics*, **9**, 703-707.
- De Jong G, Bochdanovits Z (2003) Latitudinal clines in *Drosophila melanogaster*: body size, allozyme frequencies, inversion frequencies, and insulin-signalling pathway. *Journal of Genetics*, **82**, 207-223.
- Dennill GB (1987) The biological of the weed *Acaica longifolia* by the gall wasp *Trichilogaster acaciaelongifoliae*: A study of plant-insect interaction. PhD Dissertation. University of Cape Town. South Africa.
- Dennill GB, Donnelly D, Chown SL (1993) Expansion of host-plant range of a biocontrol agent *Trichilogaster acaciaelongifoliae*. *Agriculture, Ecosystems and Environment*, **43**, 1-10.
- Dennill GB, Gordon AJ (1990) Climate-related differences in the efficacy of the Australian gall wasp (Hymenoptera: Pteromalidae) released for the control of *Acacia longifolia* in South Africa. *Environmental Entomology*, **19**, 130-136.
- Deredec A, Courchamp F (2007) Importance of the allee effect for reintroductions. *Ecoscience*, **14**, 440-451.
- de Villiers, JC (1984) The medical aspect of the Anglo-Boer War, 1899-1902. *Military History Journal*, **6**, 1-5.
- Diamond J (2002) Evolution, consequences and future of plant and animal domestication. *Nature*, **418**, 700-707.
- Di Castri F, Hansen AJ, Debusche M (1990) (eds.) Biological Invasions in Europe and the Mediterranean Basin. Kluwer Academic Publishers.
- Di Rienzo A, Peterson AC, Garza JC *et al.* (1994). Mutational processes of simple-sequence repeat loci in human populations. *Proceedings of the National Academy of Sciences of the USA*, **91**, 3166-3170.

- Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, **17**, 431-449.
- Dreier S, Vogel V, Pedersen JS, Keller L (2005) Genetic Structure and Diversity of Argentine ant (*Linepithema humile*) Populations Introduced in Chile, A Poster Presentation. In: Proceedings of the 3rd European Congress of Social Insects. St. Petersburg, Russia.
- Ellstrand NC, Schierenbeck KA (2000) Hybridization as a stimulus for evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences of the USA*, **97**, 7043-7050.
- Epperson BK (2003) Geographical Genetics. Princeton University Press, Princeton and Oxford.
- Excoffier L, Laval G, Schneider S (2005) An integrated software packages for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47-50.
- Falk DA, Knapp EE, Guerrant EO (2001) An introduction to restoration genetics. A project paper. Society for Ecological Restoration. US Environmental Protection Agency.
- Ferraris JD, Palumbi SR (1996) Molecular Zoology: Advances, Strategies & Protocols. John Wiley & Sons, Inc., New York, USA.
- Filchak KE, Kenneth E, Joseph B, Roethele LF, Jeffrey LF (2000). Natural selection and sympatric divergence in the apple maggot *Rhagoletis pomonella*. *Nature*, **407**, 739-742.
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294-299.
- Fornia A, Broderick AC, Glen F, *et al.* (2007) Genetic composition of the Ascension Island green turtle rookery based on mitochondrial DNA: implications for sampling and diversity. *Endangered Species Research*, **3**, 145-158.
- Frankham R (2005) Resolving the genetic paradox in invasive species. *Heredity*, **94**, 385.
- Frankham R, Ballou JD, Briscoe DA (2002) Introduction to Conservation Genetics. Cambridge University Press.
- Franks SJ, Richards CL, Gonzales E, Cousins JE, Hamrick JL (2004) Multiscale genetic analysis of *Uniola paniculata* (Poaceae): A coastal species with a linear fragmented distribution. *American Journal of Botany*, **91**, 345-351.

- Freckelton RP (2000) Biological control as a learning process. *Trends in Ecology & Evolution*, **15**, 263-264.
- Fredsted T, Pertoldi C, Schierup MH, Kappeler PM (2005) Microsatellite analyses reveal fine-scale genetic structure in grey mouse lemurs (*Microcebus lemurs*). *Molecular Ecology*, **14**, 2363-2372.
- Funk DJ (1999) Molecular systematics of Cytochrome oxidase I and 16S from *Neolichlamisus* leaf beetles and the importance of sampling. *Molecular Biology and Evolution*, **16**, 67-82.
- Gadagkar R (1985) Kin recognition in social insects and other animals. A review of recent findings and a consideration of their relevance for the theory of kin selection. *Proceedings of the Indian Academy of Sciences*, **94**, 587-621.
- Gardner EJ, Snustad DS (1984) Principles of Genetics, 7th ed. John Wiley and Sons.
- Gilchrist GW, Huey RB, Serra L (2001) Rapid evolution of wing size clines in *Drosophila subobscura*. *Genetica*, **112-113**, 273-286.
- Giraud T, Pedersen JS, Keller L (2002) Evolution of supercolonies: Argentine ant of southern Europe. *Proceedings of the National Academy of Sciences of the USA*, **99**, 6075-6079.
- Glaubitz JC (2004). Convert: A user-friendly program to reformat diploid genotypes data to commonly used population genetic software packages. *Molecular Ecology Notes*, **4**, 309-310.
- Goodisman MAD, Mathews RW, Crozier RH (2001) Hierarchical genetic structure of the introduced wasp *Vespula germanica* in Australia. *Molecular Ecology*, **10**, 1423-1432.
- Goolsby JA, van Klinken RD, Palmer WA (2006a) Maximising the contribution of native range studies towards the identification and prioritization of weed biological control agents. *Australian Journal of Entomology*, **45**, 276-286.
- Goolsby JA, De Barro PJ, Markinson JR, *et al.* (2006b) Matching the origin of an invasive weed for selection of a herbivore haplotype for a biological control programme. *Molecular Ecology*, **15**, 287-297.
- Gotelli JN, Colwell RK (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters*, **4**, 379-391.

- Grapputo A, Boman S, Lindström L, Lyytinen A, Mappes J (2005) The voyage of an invasive species across continents: genetic diversity of North American and European Colorado potato beetle populations. *Molecular Ecology*, **14**, 4207-4219.
- Graur, D (1985) Gene diversity in hymenoptera. *Evolution*, **39**, 190-199.
- Grevstad FS (1999) Factors influencing the chance of population establishment: Implications for release strategies in biocontrol. *Ecological Applications*, **9**, 1439-1447.
- Groves RH, Di Castri F (eds.) (1991) Biogeography of Mediterranean Invasions. Cambridge University Press.
- Gunasekera RM, Patil JG, McEnnulty FR, Bax NJ (2005) Specific amplification of mt-COI gene of the invasive gastropod *Maoricolpas rosous* in planktonic samples reveals a free-living larval life-history stage. *Marine and Freshwater Research*, **56**, 901-912.
- Hammer Ø, Harper DA, Ryan, PD (2001) PAST: Paleontological statistics software packages for education and data analysis. *Palaeontologia Electronica*, **4**, 1-9.
- Hänfling B, Carvalho G, Roland B (2002) Mt-DNA sequences and possible invasion pathways of the Chinese mitten crab. *Marine Ecology*, **238**, 307-310.
- Hardy OJ, Vekermans X (2002) SPAGeDi: A versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, **2**, 618-620.
- Hebert DNP, Cywinska A, Ball SL, de Waard JR (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London, Series B*, **270**, 313-321.
- Hedrick PW, Parker JD (1997) Evolutionary genetics and genetic variation of haplodiploids and X-linked genes. *Annual Review of Ecology and Systematics*, **28**, 55-83.
- Heller NE, Gordon DM (2006) Seasonal spatial dynamics and causes of nest movement in colonies of the invasive Argentine ant (*Linepithema humile*). *Ecological Entomology*, **31**, 499-510.
- Hengeveld R (1990) Dynamic Biogeography. Cambridge University Press.
- Hill RH, Gordon AJ, Nicer S (2000) The Potential Role of *Bruchophagus acaciae* (Cameron) (Hymenoptera: Eurytomidae) in the Integrated Control of acacia species in South Africa. In: Proceedings of the X International Symposium on Biological Control of Weeds (ed. Spencer N), pp. 919-929. Montana State University, USA.

- Hinomoto N, Higaki T, Noda T (2006) Genetic diversity in field and commercial populations of *Orius strigicollis* (Poppius) (Heteroptera: Anthocoridae) measured by microsatellite markers. *Applied Entomology and Zoology*, **41**, 499-506.
- Hirata M, Hasegawa O, Toita T, Higashi S (2008) Genetic relationships among populations of the Argentine ant *Linepithema humile* introduced into Japan. *Ecological Research*, advance online publication 09 January 2008.
- Hoffman, JH (2001) The long-leaved wattle and the bud galling wasp. The Working for Water Programme. ARC.LNR [<http://www.arc.agric.za>].
- Holway DA, Lach L, Suarez AV, Tsutsui ND, Case TJ (2002) The causes and consequences of ant invasion. *Annual Review of Ecology and Systematics*, **33**, 181-233.
- Holway DA, Suarez AV (2004) Colony structure variation and interspecific competitive ability in the invasive Argentine ant. *Oecologia*, **138**, 216-222.
- Hortal J, Borges PA, Gaspar C (2006) Evaluating the performance of species richness estimators: sensitivity to sample grain size. *Journal of Animal Ecology*, **75**, 274-278.
- Hudson RR, Slatkin M, Madison WP (1992) Estimation of levels of gene flow from DNA sequence data. *Genetics*, **132**, 583-589.
- Huey RB, Gilchrist GW, Carlson ML, Berrigan D, Serra L (2000) Rapid evolution of a geographic cline in size in an introduced fly. *Science*, **287**, 308-309.
- Hufbauer RA (2002) Evidence for nonadaptive evolution in parasitoid virulence following a biological control introduction. *Ecological Applications*, **12**, 66-78.
- Hufbauer RA, Bogdanovicz SM, Harrison RG (2004) The population genetics of a biological control introduction: mitochondrial DNA and microsatellite variation in native and introduced populations of *Aphidius ervi*, a parasitoid wasp. *Molecular Ecology*, **13**, 337-348.
- Hufbauer RA, Roderick GK (2005) Microevolution in biological control: Mechanisms, patterns, and processes. *Biological Control*, **35**, 227-239.
- Hughes JB, Hillmann JJ (2005) The application of rarefaction techniques to molecular inventories of microbial diversity. *Methods in Enzymology*, **397**, 292-307.

- Hurst GD, Jiggins FM (2005) Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: The effects of inherited symbionts. *Proceedings of Biological Society of London, Series B*, **272**, 1525-1534.
- Ingram KK (2002a) Plasticity in queen number and social structure in the invasive Argentine ant (*Linepithema humile*). *Evolution*, **56**, 2008-2016.
- Ingram KK (2002b) Flexibility in nest density and social structure in invasive populations of the Argentine ant, *Linepithema humile*. *Oecologia*, **133**, 492-500.
- Ingram KK, Gordon DM (2003) Genetic analysis of dispersal dynamics in an invading population of Argentine ants. *Ecology*, **84**, 2832-
- Jaquiéry J, Vogel V, Keller L (2005) Multilevel genetic analyses of two European supercolonies of the Argentine ant, *Linepithema humile*. *Molecular Ecology*, **14**, 589-598.
- Johnson RN, Starks PT (2004) A surprising level of genetic diversity in an invasive wasp: *Polistes dominulus* in the Northeastern United States. *Annals of Entomological Society of America*, **97**, 732-737.
- Joly S, Stevens MI, van Vuuren BJ (2007) Haplotype networks can be misleading in the presence of missing data. *Systematic Biology*, **56**, 854-862.
- Kalinowski ST (2004) Counting alleles with rarefaction: private alleles and hierarchical sampling designs. *Conservation Genetics*, **5**, 509-543.
- Kalisz S, Nason JD, Hanzawa FM, Tonsor SJ (2001) Spatial population genetic structure in *Trillium grandiflorum*: The roles of dispersal, mating, history and selection. *Evolution*, **55**, 1560-1568.
- Kaufmann B, Boomsma JJ, Passera L, Petersen KN (1992) Relatedness and inbreeding in a French population of the unicolonial ant *Iridomyrmex humilis* (Mayr). *Insectes Sociaux*, **39**, 195-213.
- Keller L, Fournier D (2002) Lack of inbreeding avoidance in the Argentine ant *Linepithema humile*. *Behavioral Ecology*, **13**, 28-31.
- Kennington WJ, Goebel J, Goldstein DB, Patridge L (2003) A Genetic Perspective of *Drosophila melanogaster* Latitudinal Body Size Clines. In: Genes in the environment (eds. Hails RS, Beringer JE, Godfrey HCJ), pp. 63-75. Blackwell Publishing.

- Kim I, Bae AS, Lee KS *et al.* (2003). Mitochondrial COI gene sequence based population genetic structure of the diamondback moth, *Plutella xylostella*, in Korea. *Korean Journal of Genetics*, **25**, 155-170.
- Knowles, L.L., 2000. Test of Pleistocene speciation in montane grasshoppers (Genus *Melanoplus*) from the sky islands of western North America. *Evolution*, **54**, 1337–1348.
- Kolbe JJ, Glor RE, Schettino LR, *et al.* (2004) Genetic variation increases during biological invasion by a Cuban lizard. *Nature*, **431**, 177-181.
- Krieger MJ, Keller K (1999) Low polymorphism at microsatellite loci in a French population of Argentine ants (*Linepithema humile*). *Molecular Ecology*, **8**, 1075-1095.
- Krieger MJ, Keller K (2000) Mating frequency and genetic structure of the Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae). *Annals of Entomological Society of America*, **97**, 1204-1215.
- Kruskal JB (1964) Non-metric multidimensional scaling: A numerical method. *Psychometrika*, **29**, 115-129.
- Laffin RD, Dosdall LM, Sperling FAH (2005) Population structure of the cabbage seedpod weevil, *Ceutorhynchus obstricus* (Marsham) (Coleoptera: Curculionidae): origins of North American introductions. *Molecular Ecology and Evolution*, **34**, 504-510.
- Lakatos F, Kovács Z, Stauffer C *et al.* (2003) The Genetic Background of Three Introduced Leafminer Moth Species- *Parectopa robiniella* Clemens 1863, *Phyllonorycter robiniella* Clemens 1859 & *Cameraria ohridella* Deschka et Dimic 1986. In: Proceedings of IUFRO Meeting on Forest Insect Population Dynamics and Host Influences (eds. Kamata N, Liebhold AM, Quiring DT, Clancy KM) pp. 67-71. Kanazawa University, Japan.
- Leberg PL (2002) Estimating allelic richness: effects of sample size and bottlenecks. *Molecular Ecology*, **11**, 2445-2449.
- Lee CE (2002) Evolutionary genetics of invasive species. *Trends in Ecology and Evolution*, **17**, 386-391.
- Lee JE, Slabber S, van Vuuren BJ, van Noort S, Chown SL (2007) Colonisation of sub-Antarctic Marion Island by a non-indigenous aphid parasitoid *Aphidius matricariae* (Hymenoptera, Braconidae). *Polar Biology*, **30**, 1195-1201.

- Liebhold AM, Tobin PC (2006) Growth of newly established alien populations: comparison of North American gypsy moth colonies with invasion theory. *Population Ecology*, **48**, 253-262.
- Liebhold AM, Tobin PC (2008) Population ecology of insect invasions and their management. *Annual Review of Entomology*, **5**, 387-408.
- Liebhold A, Bascamp J (2003) The allee effect, stochastic dynamics and the eradication of alien species. *Ecology Letters*, **6**, 133-140.
- Lloyd C, Hufbauer RA, Jackson A, Nissen SJ, Norton AP (2005) Pre- and post-introduction patterns in neutral genetic diversity in the leafy spurge gall midge, *Spurgia capitigena* (Bremi) (Diptera: Cecidomyiidae). *Biological Control*, **33**, 153-164.
- Lockwood JL, Cassey P and Blackburn T (2005) The role of propagule pressure in explaining species invasions. *Trends in Ecology and Evolution*, **2**, 223-228.
- Loeb SL (1994) An Ecological Context for Biological Monitoring. In: Biological Monitoring of Aquatic Systems (Eds. Loeb SL, Spacie A.), pp. 3-7. Levins Publishers.
- Long AD, Singh RS (1995) Molecules versus morphology: the detection of selection acting on morphological characters along a cline in *Drosophila melanogaster*. *Heredity*, **74**, 569-589.
- Louda SM, Kendall D, Connor J, Simberloff D (1997) Ecological effects of an insect introduced for biological control of weeds. *Science*, **277**, 1088-1090.
- Lowe A, Harris S, Ashton P (2004) Ecological Genetics: Design, Analysis, and Application. Blackwell Publishing.
- Luikart G, Allendorf FW, Cornuet JM, Sherwin WB (1998) Distortion of allele frequency distributions provide a test for recent population bottlenecks. *Journal of Heredity*, **89**, 238-247.
- Luruli NM (2007) Distribution and impact of the Argentine ant, *Linepithema humile* (Mayr), in South Africa. MSc thesis, Stellenbosch University.
- Macdonald AW, Jarman ML, Beeston P (1985) (Eds) Management of invasive alien plants in the Fynbos biome. South African National Scientific Programmes Report Number 111. CSIR, pp. 82-96. Pretoria.

- Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA (2000) Biotic invasions: causes, epidemiology, global consequences and control. *Ecological Applications*, **10**, 689-710.
- Manongi FS, Hoffman JH (1995) The incidence of parasitism in *Trichilogaster acaciaelongifoliae* (Frogatt) (Hymenoptera: Pteromalidae), a gall-forming biological control agent of *Acacia longifolia* (Andr.) WILD (Fabaceae) in South Africa. *African Entomology*, **3**, 147-151.
- Markin GP (1969) Foraging behaviour of the Argentine ant in California Citrus Grove. *Journal of Economic Entomology*, **63**, 740-744.
- Markin GP (1970) Seasonal life cycle of the Argentine ant, *Iridomyrmex humilis*, (Hymenoptera: Formicidae). *Annals of Entomological Society of America*, **63**, 1238-1242.
- Maynard Smith J, Haigh J (1974) The hitch-hiking effect of a favourable gene. *Genetic Research*, **23**, 23-35.
- McDonald DB, Potts WK (1997) Microsatellite DNA as a Genetic Marker at Several Scales. In: Avian Molecular Evolution and Systematics (ed. Mindell D), pp. 29-49. Academic Press, New York.
- McEvoy PB (1996) Host specificity and biological pest control. *Bioscience*, **46**, 401-405.
- McFadden CS, Aydin KY (1996) Spatial autocorrelation analysis of small scale genetic structure in a clonal soft coral with limited larval dispersal. *Marine Biology*, **126**, 215-224.
- McGeoch MA, Wossler TC (2000) Range expansion and success of the weed biocontrol agent *Trichilogaster acaciaelongifoliae* (Frogatt) (Hymenoptera: Pteromalidae) in South Africa. *African Entomology*, **8**, 273-280.
- Meixner MD, McPherson BA, Silva JG, Gasparich GE, Sheppard WS (2002) The Mediterranean fruit fly in California: evidence for multiple introductions and persistent populations based on microsatellite and mitochondrial DNA variability. *Molecular Ecology*, **11**, 891-899.
- Memmott J, Craze PG, Harman HM, Syrett P, Fowler SV (2005) The effect of propagule size on the invasion of an alien insect. *Journal of Animal Ecology*, **74**, 50-62.
- Menke SB, Holway DA (2006) Abiotic factors control invasion by Argentine ants at the community scale. *Ecology*, **88**, 3164-3174.

- Merrel DJ (1981) Ecological Genetics. Longman, London.
- Mescher MC, Ross KG, Shoemaker DD, Keller L, Krieger MJB (2003) Distribution of the two social forms of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae) in the native South America range. *Annals of the Entomological Society of America*, **96**, 810-817.
- Miller III DG, Crespi B (2003) The evolution of inquilinism, host-plant use and mitochondrial substitution rates in *Tamalia* gall aphids. *Journal of Evolutionary Biology*, **16**, 731-743.
- Miura O (2007) Molecular genetic approaches to elucidate the ecological and evolutionary issues associated with biological invasions. *Ecological Research*, **22**, 876-883.
- Money HA, Cleland EE (2001) The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences of the USA*, **98**, 5446-5451.
- Moran PAP (1950) Notes on continuous stochastic phenomena. *Biometrika*, **37**, 17-23.
- Muirhead JR, Gray DK, Kelly DW *et al.* (2008) Identifying the source of species invasions: sampling intensity vs. genetic diversity. *Molecular Ecology*, **17**, 1020-1035.
- Myburgh M, Chown SL, Daniels SR, van Vuuren BJ (2007) Population structure, propagule pressure and conservation biogeography in the Sub-Antarctic: Lessons from indigenous and invasive springtails. *Diversity and Distributions*, **13**, 143-154.
- Myers, J. H. (2000). What can we learn from biological control failures? In: Proceedings of the X International Symposium on Biological Control of Weeds (ed. Spencer NR), pp. 151-154. Montana State University, USA.
- Nauman JS, Zungoli PA, Benson EP (2004) Argentine ants. Entomology Insect Information Series. Cooperative Extension Services. Clemson University.
- Nielsen R (2001) Statistical tests of selective neutrality in the age of genomics. *Heredity*, **86**, 641-647.
- Noble NS (1940). *Trichilogaster acaciaelongifoliae* (Frogatt) (Hymenoptera, Chalcidoidea), a wasp causing galling of the flower-buds of *Acacia longifolia* WILLD., *A. floribunda* SIEBER & *A. sophorae* R. BR. *Transactions of the Entomological Society of London*, **90**, 13-38.
- Olson LJ (2006) The economics of terrestrial invasive species: A review of the literature. *Agricultural and Resource Economics Review*, **35**, 178-194.

- Packer L, Owen R (2001) Population genetic aspects of pollinator decline. *Conservation Ecology*, **5**, 163-198.
- Palmer MW (1990) The estimation of species richness by extrapolation. *Ecology*, **71**, 1195-1198.
- Palmer MW (1991) Estimating species: the second order Jackknife reconsidered. *Ecology*, **72**, 1512-1513.
- Pappert RA, Hamrick JL, Donovan LA (2000) Genetic variation in *Pueraria lobata* (Fabaceae), an introduced, clonal, invasive plant of the Southeastern United States. *American Journal of Botany*, **87**, 1240-1245.
- Parker IM, Reichard SH (1998) Critical issues in invasion biology for conservation science. In: Conservation Biology (eds. Fiedler PL, Kareiva, PM), pp.283-305. Chapman Hall.
- Parsons PA (1983) The Evolutionary Biology of Colonizing Species. Cambridge University Press.
- Passera L, Keller L (1994) Mate availability and male dispersal in Argentine ant *Linepithema humile* (Mayr) (*Iridomyrmex humilis*). *Animal Behavior*, **48**, 361-369.
- Pedersen, J. S., Krieger, M. J. B., Vogel, V., Giraud, T. & Keller, L. (2006). Native supercolonies of unrelated individuals in the invasive Argentine ant. *Evolution*, **60**, 782-791.
- Petit RJ, Bialozyt R, Garnier-Géré P, Hampe A (2004) Ecology and genetics of tree invasions: from recent introductions to quaternary migrations. *Forest Ecology and Management*, **197**, 117-137.
- Pickett KM, Wenzel JW (2000) High productivity in haplometrotic colonies of the introduced paper wasp *Polistes dominulus* (Hymenoptera: Vespidae; Polistinae). *Journal of the New York Entomological Society*, **108**, 314-325.
- Pimental D, Lach L, Zuniga R, Morrison D (2000) Environmental and economic costs of non-indigenous species in the United States. *Bioscience*, **50**, 53-65.
- Piry S, Luikart G, Cornuet JM (1999) Bottleneck: A computer program to calculate F-statistics. *Heredity*, **86**, 485-486.
- Posada D, Crandall KA (2001) Selecting the best-fit model of nucleotide substitution. *Systematic Biology*, **50**, 580-601.

- Prins AJ (1978) Hymenoptera. In: Werger, M. J. A. (Ed.). Biogeography and Ecology of southern Africa. Junk, The Hague, The Netherlands.
- Puillandre N, Dupas S, Dangles O, Zeddam JL, Capdevielle-Dulac C, Barbin K, Torres-Lequizarmon M, Silvain JF (2008) Genetic bottleneck in invasive species: the potato tuber moth adds to the list. *Biological Invasions*, **10**, 319-333.
- Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution*, **43**, 258-275.
- Raymond M, Rousset F (1995) Genepop1.2: Population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248-249.
- Reusch TBH, Hukriede W, Stam WT, Olsen JL (1999) Differentiation between clonal growth and limited gene flow using spatial autocorrelation of microsatellites. *Heredity*, **83**, 1120-126.
- Reuter M, Balloux F, Lehmann L, Keller L (2001) Kin structure and queen execution in the Argentine ant *Linepithema humile*. *Journal of Evolutionary Biology*, **14**, 954-958.
- Richardson MD, Pyšek, P (2006) Plant invasions: Merging the concepts of species invasions and community invasibility. *Progress in Physical Geography*, **30**, 409-431.
- Roderick G K, Navajas M (2003). Genes in new environments: Genetics and evolution in biological control. *Nature Reviews Genetics*, **4**, 889-899.
- Roff D (2003) Evolutionary danger for rainforest species. *Science*, **301**, 58-59.
- Ros VID, Breeuwer JAJ (2007) Spider mite (Acari: Tetranychida) mitochondrial COI phylogeny reviewed: host plant relationships, phylogeography, reproductive parasites and barcoding. *Experimental Applications and Acarology*, **42**, 239-262.
- Ross KG (2001) Molecular ecology of social behaviour: analysis of breeding systems and genetic structure. *Molecular Ecology*, **10**, 265-284.
- Roura-Pascual N, Suarez AV, Gómez C *et al.* (2004). Geographical potential of Argentine ants (*Linepithema humile*) in the face of global climate change. *Proceedings of the Royal Society of London, Series B*, **271**, 2527-2534.
- Rozas J, Sánchez-DelBarrio JC, Messager X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, **19**, 2496-2497.

- Sakai AK, Allendorf FW, Holt JS *et al.* (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics*, **32**, 305-332.
- Schäfer MA, Hille A, Uhl GB (2001) Geographical patterns of genetic subdivision in the cellar spider *Pholeus phalangioides* (Araneae). *Heredity*, **86**, 94-102.
- Scheffer SJ, Lewis ML, Joshi RC (2006) DNA barcoding applied to invasive leafminer (Diptera: Agromyzidae) in the Philipines. *Annals of Entomological Society of America*, **99**, 304-310.
- Shoemaker DD, Keller G, Ron KG (2003) Effects of *Wolbachia* on mt DNA variation in two fire ant species. *Molecular Ecology*, **12**, 1757-1771.
- Silverman J, Brightwell RJ (2008) The Argentine ant: Challenges in managing an invasive unicolonial pest. *Annual Review of Entomology*, **53**, 231-252.
- Skaife SH. (1953) African Insect Life. Longmans Green & Co. LTD. Cape Town.
- Slade RW, Moritz C (1998) Phylogeography of *Bufo marinus* from its natural and introduced ranges. *Proceedings of the Royal Society of London, Series B*, **265**, 760-777.
- Slatkin M (1985) Rare alleles as indicators of gene flow. *Evolution*, **39**, 53-65.
- Slatkin M, Arter HE (1991) Spatial autocorrelation methods in population genetics. *The American Naturalist*, **138**, 499-517.
- Slatkin M, Hudson RR (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics*, **129**, 555-562.
- Slingsby P, Bond WJ (1981) Ants-Friends of the fynbos. *Veld and Flora*, **67**, 39-45.
- Slingsby P, Bond WJ (1983) Of ants and proteas. *African Wildlife*, **36**, 104-107.
- Smith, PT (2005) Mitochondrial DNA variation among populations of the glassy-winged sharpshooter, *Homalodisca coagulata*. *Journal of Insect Science*, **5**, 41-48.
- Sokal RR, Oden NL (1978a) Spatial autocorrelation in biology 1 Methodology. *Biological Journal of the Linnean Society*, **10**, 199-228.
- Sokal RR, Oden NL (1978b) Spatial autocorrelation in biology 2: some implications and four applications of evolutionary interests. *Biological Journal of the Linnean Society*, **10**, 229-249.

- Sokal RR, Rohlf FJ (1995) Biometry: The principles and practice of statistics in biological research. 3rd ed. W. H. Freeman, New York.
- Stone G N, Atkinson RJ, Brown G, Rokas A, Csóka G (2003) The population genetic consequences of range expansion: Oak gall wasps as a model system. In: Genes in the Environment (eds. Hails RS, Bering JE, Godfrey, HCJ), pp. 46-62. Blackwell and the British Ecological Society.
- Suarez AV, Holway DA, Tsutsui ND (2008) Genetics and behaviour of a colonizing species: The invasive Argentine ant. *The American Naturalist*, **172**, S72-S84.
- Suarez AV, Tsutsui ND (2008) The evolutionary consequences of biological invasions. *Molecular Ecology*, **17**, 351-360.
- Suarez AV, Tsutsui ND, Holway DA, Case TJ (1999) Behavioural and genetic differentiation between native and introduced populations of the Argentine ant. *Biological Invasion*, **1**, 43-53.
- Suarez AV, Holway DA, Case TJ (2001) Patterns of spread in biological invasions dominated by long-jump dispersal: Insights from Argentine ants. *Proceedings of the National Academy of Sciences of the USA*, **98**, 1095-1100.
- Sugiyama T (2000) Invasion of Argentine ant, *Linepithema humile*, into Hiroshima Prefecture, Japan. *Japanese Journal of Applied Entomology and Zoology*, **44**, 127-129.
- Swofford D (2003) PAUP*4.0b10: Phylogenetic analysis using parsimony (*and other methods). Sinauer, Sunderland, MA.
- Therriault TW, Orlova MI, Docker MF, MacIsaac HJ, Heath DD (2005) Invasion genetics of freshwater mussel *Dreissia rostriformis bugensis* in Eastern Europe: high gene flow and multiple introductions. *Heredity*, **95**, 16-23.
- Thomas ML, Payne-Makrisa CM, Suarez AV, Tsutsui ND, Holway DA (2006) When supercolonies collide: territorial aggression in an invasive and unicolonial social insect. *Molecular Ecology*, **15**, 4303-4315.
- Thomas ML, Payne-Makrisa CM, Suarez AV, Tsutsui ND, Holway DA (2007) Contact between supercolonies elevates aggression in Argentine ants. *Insects Society*, **54**, 225-233.

- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins, DG (1997). The Clustal_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, **25**, 4876-4882.
- Tsutsui ND, Case TJ (2001) Population genetics and colony structure of the Argentine ant (*Linepithema humile*) in its native and introduced ranges. *Evolution*, **55**, 976-985.
- Tsutsui ND, Suarez AV (2003) The colony structure and population biology of invasive ants. *Conservation Biology*, **17**, 48-58.
- Tsutsui ND, Suarez AV, Holway DA, Case TJ (2000) Reduced genetic variation and the success of an invasive species. *Proceedings of the National Academy of Sciences of the USA*, **97**, 5954-5953.
- Tsutsui ND, Suarez AV, Holway DA, Case TJ (2001) Relationships among native and introduced populations of the Argentine ant (*Linepithema humile*) and the source population of the introduced populations. *Molecular Ecology*, **10**, 2151-2161.
- Tsutsui ND, Suarez AV, Grosberg RK (2003) Genetic diversity, asymmetrical aggression, and recognition cues in a wide spread invasive species. *Proceedings of the National Academy of Sciences of the USA*, **100**, 1078-1083.
- van Driesche RG, Bellows TS (1996) Biological control. Chapman & Hall, New York.
- van Kleunen M, Johnson SD (2005) Testing for ecological and genetic allee effects in the invasive shrub *Senna didymobotrya* (Fabaceae). *American Journal of Botany*, **92**, 1124-1130.
- van Klinken RD, Edwards OR (2002) Is host-specificity of weed biological control agents likely to evolve rapidly following establishment? *Ecology Letters*, **5**, 590-596.
- van Oosterhout C, William F, Hutchinson DP, Wills M, Shipley P (2004) Micro-checker: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535-538.
- Vega SJ, Rust MK (2001) The Argentine ant- a significant invasive species in agricultural, urban and natural environments. *Sociobiology*, **37**, 3-25.
- Vernon PV (1985) "The Royal New South Wales Lancers 1885-1985" The Royal New South Wales Lancers Centenary Committee, Parramatta Australia.

- Walters AC, Mackay DA (2004) Comparisons of upper thermal tolerance between the invasive Argentine ant (Hymenoptera: Formicidae) and two native Australian ant species. *Annals of Entomological Society of America*, **97**, 971-975.
- Ward DF, Beggs JR, Clout MN, Harris RJ & O'Connor S (2006) The diversity and origin of exotic ants arriving in New Zealand via human-mediated dispersal. *Diversity & Distributions*, **12**, 601-609.
- Ward M, Johnson SD (2005) Pollen limitation and demographic structure in small fragmented populations of *Brunsvigia radulosa* (Amaryllidaceae). *Oikos*, **108**, 253-262.
- Wetterer J, Wetterer A (2006) A disjunct Argentine ant metacolony in Macaronesia & South-western Europe. *Biological Invasions*, **8**, 1123-1129.
- Wild AL (2004) Taxonomy and distribution of the Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae). *Annals of Entomological Society of America*, **97**, 1204-1215.
- Witt ARR, Giliomee JH (1999) Soil-surface temperature at which six species of ants (Hymenoptera: Formicidae) are active. *African Entomology*, **7**, 161-164.
- Wolstenholme DR (1992) Animal mitochondrial DNA: structure and evolution. *International Review of Cytology*, **141**, 173-216.
- Wrigley J, Fagg M (1996) Australian native plants, 4th ed. Reed Books Pty Ltd.
- Zar JH (1999) Biostatistical analysis. 4th ed. Prentice Hall, Upper Saddle River, NJ.
- Zayed A, Constantin ŞA, Packer L (2007) Successful Biological Invasion despite a Severe genetic Load. *PLoS ONE* **2**:e868.
- Zee J, Holway DA (2006) Nest raiding by the invasive Argentine ant on colonies of the harvester ant, *Pogonomyrmex subnitidus*. *Insectes Sociaux*, **53**, 161-167.

Appendix

Table 11 Allele frequencies of Argentine ants in South Africa

Pop	Blom	Bell	Clan	Deme	Elim	Lain	Nige	Nieu	Port	Rich	Stel	Spri	Stor	Tulb	Unio	Upin	AIN	LaGr	BlomI	LaBr	BeBa	Cale	Koge
A1	0.6	0.5	0.8	0	0.45	0.3	0.1	0.8	0	0.5	0.35	0.35	0.75	0.45	0.3	0.4	0.65	0.45	0	0.45	0.85	0.5	0.4
A2	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A3	0	0	0	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A4	0	0	0	0.1	0	0	0	0	0.45	0	0	0.05	0	0	0	0	0	0	0.4	0	0	0	0
A5	0	0	0	0.3	0	0	0	0	0.25	0	0	0.05	0	0	0	0	0	0	0.15	0	0	0	0
A6	0.3	0.5	0.2	0	0.55	0.7	0.9	0.2	0	0.5	0.65	0.55	0.15	0.55	0.7	0.6	0.35	0.55	0	0.55	0.15	0.5	0.6
A7	0	0	0	0.45	0	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0.4	0	0	0	0
A8	0	0	0	0.05	0	0	0	0	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A9	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0.05	0	0	0	0
A10	0	0	0	0	0	0	0	0	0	0	0	0	0.05	0.05	0	0	0	0	0	0	0	0	0
A11	0.05	0	0.1	0	0.05	0.25	0.05	0.3	0	0.25	0.1	0.2	0.2	0.05	0.15	0.25	0.55	0.05	0	0	0.15	0.1	0.65
A12	0.35	0.1	0.45	0.4	0.25	0.05	0.6	0.15	0.35	0.1	0.2	0.1	0.15	0.35	0.4	0.35	0.2	0.05	0.2	0.35	0.4	0.25	0.2
A13	0	0.1	0	0	0	0	0	0	0	0	0	0	0.05	0.05	0	0	0	0	0	0	0	0.05	0
A14	0	0.1	0	0	0	0	0.05	0.15	0	0	0	0	0.05	0	0.1	0	0	0	0	0	0	0	0.1
A15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.05	0
A16	0.15	0.15	0.1	0.6	0.2	0.1	0	0	0.6	0.1	0.05	0.1	0	0.1	0	0.05	0	0	0.8	0.05	0	0	0
A17	0.45	0.55	0.2	0	0.45	0.6	0.3	0.4	0	0.55	0.65	0.55	0.5	0.4	0.35	0.35	0.25	0.9	0	0.6	0.45	0.55	0.05
A18	0	0	0.15	0	0.05	0	0	0	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A19	0	0	0	0	0	0	0	0	0	0	0	0.05	0	0	0	0	0	0	0	0	0	0	0
A20	0	0	0	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A21	0	0	0	0.05	0	0	0	0	0.05	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0
A22	0.55	0.4	0.35	0.9	0.5	0.4	0.15	0.3	0.9	0.15	0.45	0.15	0.35	0.4	0.15	0.55	0.4	0.1	0.85	0.25	0.35	0.45	0.15
A23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.05	0	0	0	0	0	0	0	0
A24	0.35	0.35	0.3	0	0.3	0.45	0.25	0.45	0	0.4	0.15	0.5	0.2	0.45	0.05	0.35	0.2	0.2	0	0.35	0.1	0.25	0.75
A25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.05	0	0	0	0	0
A26	0	0	0	0	0	0	0.05	0	0.05	0	0	0	0	0	0	0	0.25	0.1	0	0	0	0	0
A27	0	0	0	0	0	0	0.05	0	0	0	0.05	0	0	0	0	0	0	0	0	0	0	0	0
A28	0.1	0.2	0	0	0.1	0.15	0.5	0.2	0	0.4	0.25	0.35	0.15	0.15	0.5	0.1	0.15	0.35	0	0.25	0.15	0.15	0.05
A29	0	0	0	0	0	0	0	0	0	0.05	0	0	0.1	0	0	0	0	0	0	0.05	0	0	0
A30	0	0	0.2	0	0.05	0	0	0.05	0	0	0.05	0	0.1	0	0.05	0	0	0.05	0.05	0	0.3	0.1	0
A31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.05	0	0	0	0	0	0	0	0
A32	0	0	0.1	0	0	0	0	0	0	0	0.05	0	0.05	0	0.05	0	0	0.05	0	0	0	0	0
A33	0	0.05	0.05	0	0.05	0	0	0	0	0	0	0	0.05	0	0.05	0	0	0.1	0	0.1	0.1	0.05	0.05
A34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.05	0	0	0	0	0	0	0	0
A35	0.05	0.2	0.1	0	0.05	0.25	0.05	0.15	0	0.1	0.1	0.2	0.05	0.05	0.2	0.1	0.15	0	0.05	0.3	0.35	0.15	0.15
A36	0	0.05	0.35	0	0.1	0.2	0.4	0.2	0	0.3	0.15	0.15	0.5	0.2	0.2	0.05	0.05	0.3	0	0.25	0.4	0.25	0.35
A37	0	0	0	0.05	0.05	0	0	0	0.35	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A38	0.1	0.1	0.05	0	0	0.1	0.35	0.05	0.1	0.05	0.2	0	0	0.25	0.1	0.2	0	0.15	0	0.05	0	0.05	0.1
A39	0.1	0.05	0	0.95	0.15	0	0	0	0.5	0.05	0.05	0.05	0	0.05	0.1	0.1	0.05	0	1	0	0.15	0	0.05
A40	0.15	0.15	0.2	0	0.25	0.15	0	0.45	0.05	0	0.1	0.2	0.25	0	0.35	0.05	0.25	0.1	0	0.3	0.05	0.15	0.1
A41	0.4	0.05	0.3	0	0.2	0.2	0.15	0	0	0.4	0.3	0.45	0	0.15	0.2	0.3	0.4	0.15	0	0.3	0.1	0.15	0.2
A42	0.2	0.25	0	0	0.2	0.1	0.05	0.1	0	0.05	0.1	0.05	0.05	0.25	0	0.1	0.15	0	0.05	0	0.05	0.05	0.05
A43	0	0	0	0	0	0	0	0.05	0	0.05	0	0	0	0.05	0	0	0	0	0	0	0	0	0
A44	0	0.15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A45	0	0	0	0	0	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A46	0	0.05	0	0	0	0	0	0	0	0	0	0	0.05	0.05	0	0	0	0	0	0	0	0	0
A47	0	0	0	0.9	0	0	0	0	0.9	0	0	0	0	0	0	0	0.25	0	0.95	0	0	0	0
A48	0.65	0.6	0.8	0	0.8	0.9	0.45	0.8	0	0.75	0.85	0.85	0.55	0.55	0.75	0.65	0.6	0.95	0	0.9	0.85	1	0.95
A49	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A50	0	0	0	0	0	0	0	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A51	0.15	0.2	0.2	0.1	0.15	0.05	0.2	0.15	0.1	0.15	0.15	0.1	0.3	0.2	0.2	0.15	0	0	0	0	0	0	0
A52	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.05	0	0	0	0	0
A53	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A54	0	0	0	0	0	0	0.15	0	0	0	0	0	0	0.05	0	0	0	0	0	0.05	0	0	0
A55	0	0	0	0	0	0	0.05	0	0	0.05	0	0	0.05	0.05	0	0	0	0	0	0	0.05	0	0.05
A56	0.05	0.1	0	0	0.05	0	0.15	0	0	0.05	0	0.05	0.05	0.1	0.05	0.2	0.1	0.05	0	0.05	0.1	0	0
A57	0	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.05	0	0	0	0	0	0
A58	0	0	0	0	0.05	0	0	0	0	0	0.05	0.05	0.1	0.05	0.05	0	0	0	0	0	0	0	0
A59	0.85	0.85	0.8	0.6	0.75	0.95	0.85	0.85	0.7	0.85	0.7	0.85	0.8	0.85	0.85	0.8	0.9	1	0.95	0.9	1	1	1
A60	0.15	0.15	0.2	0.4	0.2	0.05	0.15	0.15	0.3	0.15	0.25	0.1	0.1	0.1	0.1	0.2	0.1	0	0.05	0.1	0	0	0